

CELL CULTURE CATALOGUE

Classical Media
Special Media
Salt Solutions
Buffers
Reagents
Supplements
Sera
Technical Information



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BioConcept CELL CULTURE CATALOGUE



Dear Customer

We are proud to present our catalogue, which provides you with a concise overview of our company and our products.

For our catalogue, we have chosen “life in the ocean” as the theme. The oceans were the original incubator of life and provide a complex and delicate balance, which supports the diversity of life. With our cell culture products, BioConcept Ltd. seek to emulate nature by providing the optimal medium to support cell cultivation.

Thank you for using our products and services,

BioConcept Ltd.

Martin Howald

CEO/President

Who we are

BioConcept Ltd. is supplying high-tech tools and products to the biological research community since 1978.

We are a dedicated system supplier, providing our customers with a comprehensive product portfolio and customized solutions.

Going ahead

Over the years we have become a leading supplier and service partner for numerous respected pharmaceutical and academic institutions in Switzerland and around the world.

With our new facilities, and our convenient location near Basel airport, we aspire to expand further internationally while also serving local biological researchers. We shall continue to listen to the needs of the market and be flexible when meeting them.

Iceland

Canada
USA

I Sub-Saharan Africa

Austria
Czech Republic
Denmark
Estonia
Finland
Germany
Hungary
Italy

Latvia
Lithuania
Netherlands
Poland
Portugal
Slovakia
Sweden

China
India
Japan
Singapore
Taiwan

65%
Sales international

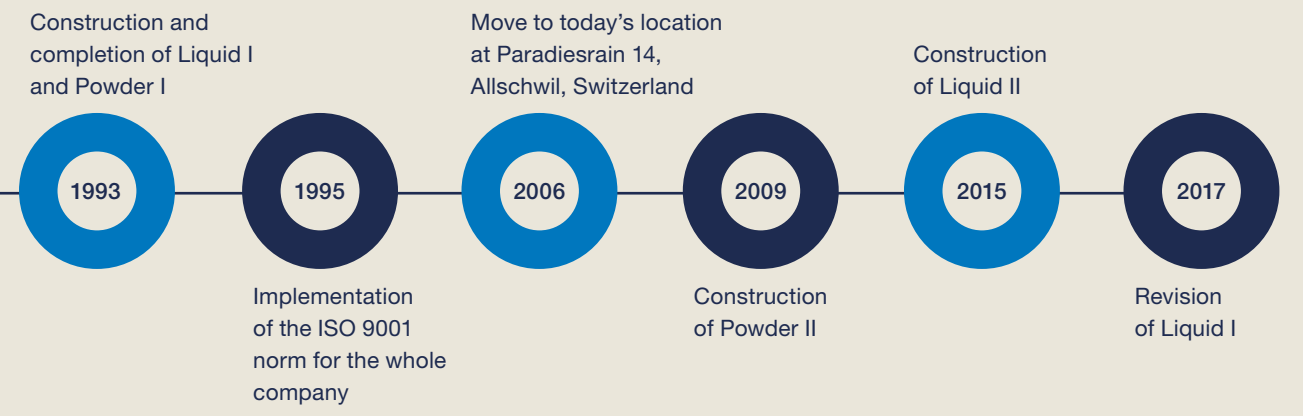
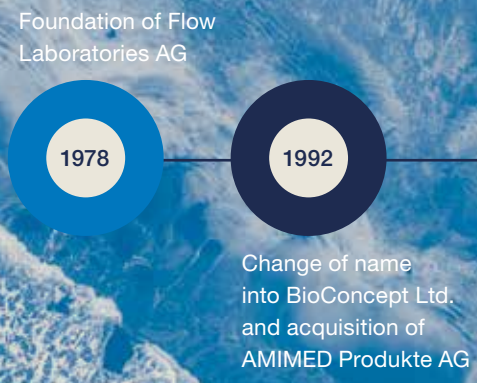
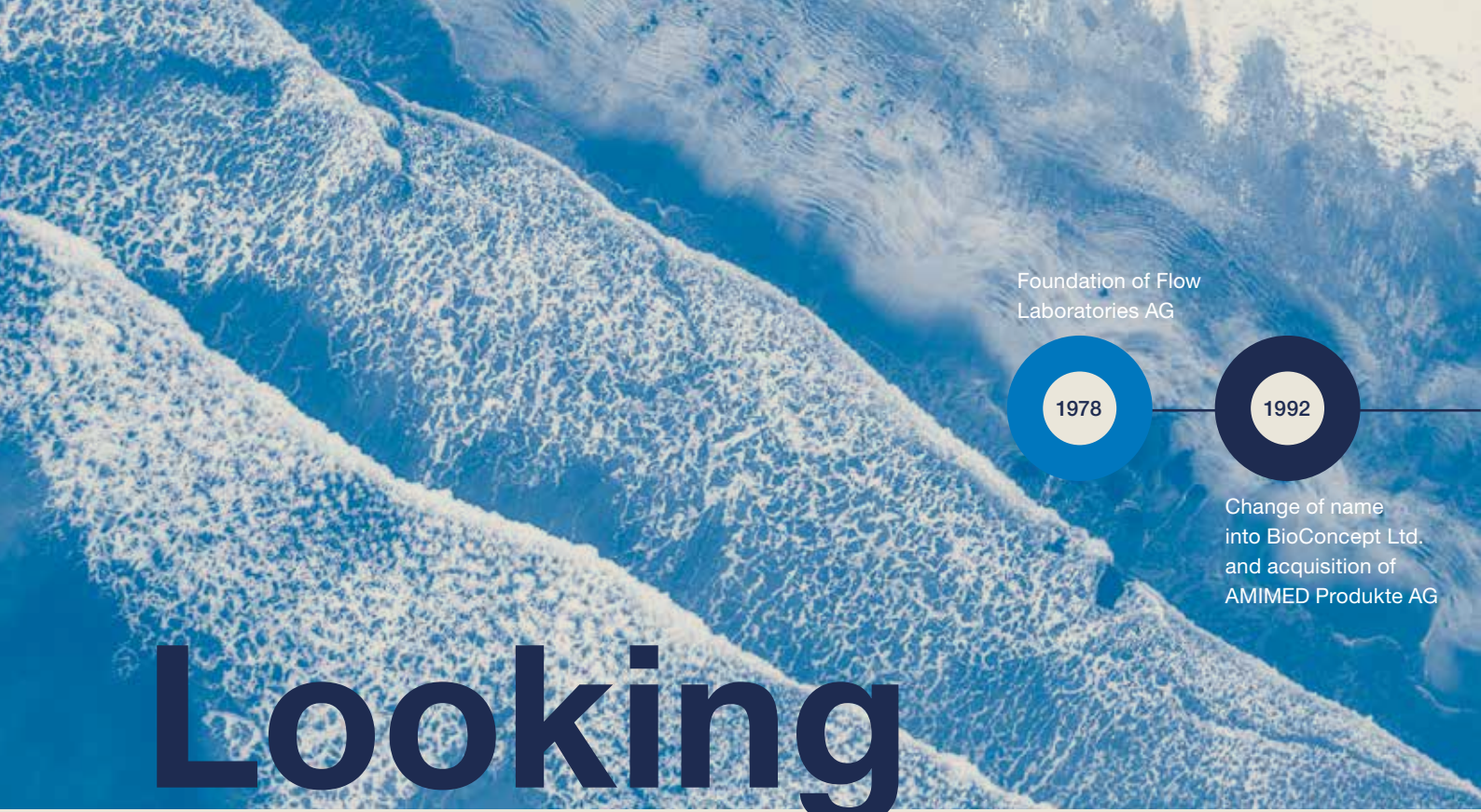
2018

35%
Sales Switzerland

5%
Sales international

1995

95%
Sales Switzerland



Looking back

At BioConcept Ltd. we are proud of the progress we have made since our foundation in 1978.

We have been producing within a certified quality management system since 1995 and our new cell culture and sterile liquid production plant has vastly improved our already high standards. BioConcept Ltd.'s expansion into the tissue culture market in 1992 allowed us to meet the needs of the sophisticated and evolving pharmaceutical and bio-pharmaceutical markets.

Giving back

We believe that we can only operate well in a healthy society and environment.

Hence we commit ourselves to support various cultural and educational programs.



Support of the Swiss research community

PhD- and postdoctoral retreats are events aiming to foster scientific exchange and progress.

As scientific areas are no longer stand alone research fields and interdisciplinary research is the new gold standard, such retreats encourage the collaboration between the PhD students and postdoctoral Fellows. They have the opportunity to share their work, talk about science and build new relationships.

Ozeanium Basel

At the Ozeanium, which will be part of the Basel Zoo, visitors dive into fascinating underwater worlds and learn more about the complex blue ecosystem that covers our planet. On an area of approximately 10 000 m², 40 aquariums accommodate sharks, rays, penguins, corals and many more species in over 4.6 Mio. litres of water. Several thousands of animals from all over the globe convey the size of the ocean, its diversity, its beauty, but also its fragility and its threats. The entire Ozeanium revolves around the subject of resources and sustainability, turning its audience into experts for a world that needs to be valued and protected. [1](#)

Tembea

Tembea is the name of the new elephant enclosure in the Basel Zoo. After just over three years of construction, it opened its doors for the public in 2017. At over 5 000 m², the new facility is two and a half times the size of the old one. The public can view the elephants in the open enclosure and the near-natural savannah landscape. In addition to African elephants, harvester ants, brown rats and a few species of fish live in the Elephant house and the enclosure provides nesting areas for birds and accommodation for bats. [2](#)

Isele

In Isele, a small village in East African Tanzania, a new village centre is built with the help of several sponsors. An administration building with offices and a meeting room, such as a shop and an Internet café are already completed. Furthermore, there will be an economy building with workshops and a corn mill will complement the new compound. Thus, on the one hand, economic activities are promoted and, on the other hand, already existing organizations and social structures are consolidated locally. In the future, further buildings are to be built in the surrounding area and incorporated into a village structure. [3](#)

Jazz and Blues Events, Marabu Blues Night

Built as a school, turned into a cinema and now a cultural institution in Gelterkinden, the Marabu has a long and fascinating history and a unique ambience. Today, the independent non-profit organization Kultur Marabu Gelterkinden puts together a diverse program and the annual Blues Night is definitely a highlight in the agenda. The live music event is dedicated to promoting blues music in Switzerland and presents local bands.

Frauenplus

Frauenplus Baselland is an independent non-profit women's organization dedicated to helping women and their families in difficult situations. The organization's objective is to alleviate hardship fast and in a straightforward manner.



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About us

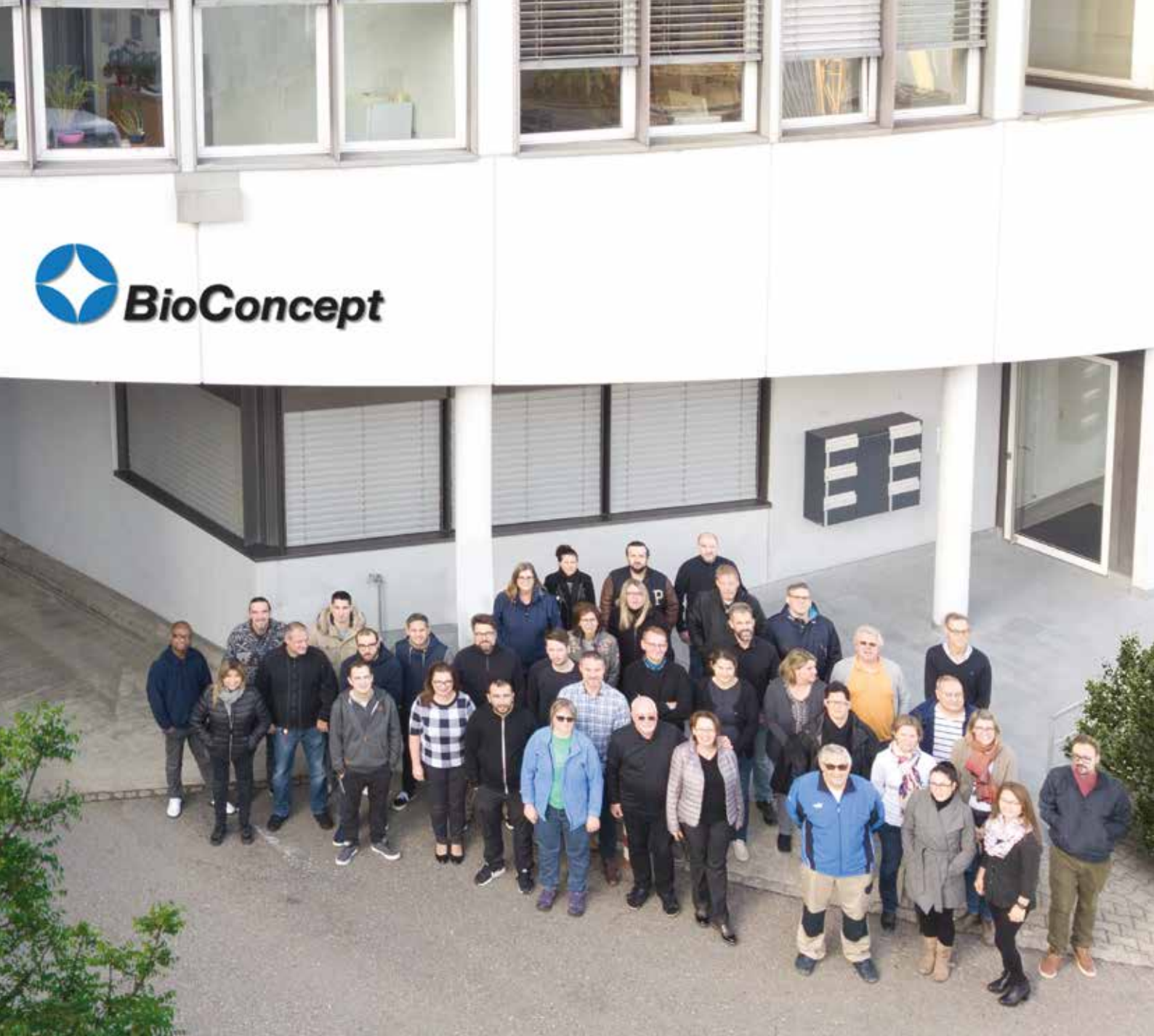
Infrastructure

Products

About
BioConcept Ltd.

Great Barrier reef

The great barrier reef, located north east of Australia, contains an immense variety of life. It is inhabited by 400 types of coral and 1500 known species of fish, which is around 10 % of all fish species in the world. The coral system is in fact made up of 2900 individual reefs, covers an area of 344400 square meters, and is the world's largest living structure. Sadly, it is currently facing some of the greatest threats in its life span, as recent heatwaves have bleached 90 % of the corals in some places. There is some hope, however. Scientists around the world are constantly finding new ways to support the reef and help it recover. Furthermore, the Australian government has recently unveiled a £60 million protection plan for the reef as a response to its current problems.



At BioConcept Ltd. we are proud of our dynamic team and the available know-how we like to share with our partners. We are happy to move on together.

About us

BioConcept Ltd. is a privately owned company with more than 40 years of experience in serving the biological community. We strive to combine this experience with an innovative spirit to support our customer's needs with the best possible solutions.

To do so, BioConcept Ltd. establishes close relationships with its customers to be able to respond to their wishes. To guarantee that our products are of the highest standard, BioConcept Ltd. has been producing within a certified quality management system (ISO 9001) since 1995. The current ISO standard is 9001:2015. Our SQS certificate can be downloaded at www.bioconcept.ch.

Please contact our specialists if you would like to discuss possibilities in further detail:

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info@bioconcept.ch



1

- 1 The 5000 L state-of-the-art media tank is linked to a cleaning- and sterilization-in-place system (CIP/SIP system).
- 2 Powder II with a batch size up to 800 kg.
- 3 Liquid I filling line for special media in the ISO 5 cleanroom.
- 4 In-house QC (Quality Control) ensures high level of quality.

2



3



4

Infrastructure

BioConcept Ltd. is currently running two independent liquid media production facilities (Liquid I and Liquid II) and two independent powder media production facilities (Powder I and Powder II).

Both are equipped with qualified ISO 5 (GMP A) cleanrooms. Liquid I is used for production of special media and small batch sizes from 5 L up to 1500 L. With Liquid I we are able to handle a broad range of container sizes from 1 ml up to 500 L, giving us a great deal of flexibility to fulfil our customers requests. Liquid II is the fully automated production facility of BioConcept Ltd., which went online in 2015. It is exclusively used for Animal Component Free (ACF) production. The plant has been designed to maximize efficiency, sustainability and productivity. Its layout as well as its equipment is in accordance with GMP guidelines, e.g. air and water preparation facility, calibrated and qualified instruments, validated processes, fully integrated Standard Operating Procedures (SOPs) and comprehensive documentation of production. The state-of-the-art air processing system provides us with the optimal conditions required for sterile liquid production. We constantly monitor and control the pressure, temperature, humidity and particle count in the air and we regularly disinfect our facilities using H₂O₂. The water preparation facilities are specifically engineered to efficiently generate the highest standard Water For Injection (WFI) available. This gives us the capability to generate 5000 L of media a day, allowing us to meet the rising demands from our customers. For the preparation of the bottles we use a cleanroom robot to minimize the particle count. All further steps, including sealing and labelling, are also performed automatically. In summary, the plant has been designed to create an environment that upholds the highest degree of sterility possible, thereby ensuring a sterile final product.

BioConcept Ltd. runs two powder media plants (Powder I and II) with a capacity of 100 kg/day and 800 kg/day, respectively. The impact mills produce powder with an average grain size of < 20 µm. The plants are made for batch sizes from 2 kg up to 800 kg, which can be filled into various container sizes, in accordance to the customers needs. The advantages for the customer of our powder plants are the high homogeneity of the product, with a low particle size, a low risk of cross-contamination and the easy and safe handling.

In addition to these production facilities, BioConcept Ltd. guarantees the high quality of the products with its in-house quality control (QC). BioConcept Ltd. performs tests for sterility, pH-value, osmolality, cell growth, endotoxin levels and conductivity. For powder formulations we also test the bioburden. Further tests, if required, are outsourced to certified analytical laboratories.



1

- 1 The robotic arm plays a crucial role in the automatic filling line in LQII.
- 2 The automated filling process ensures reliable sterility.
- 3 In LQII containers up to 1 000 L are easily handled.

2



3



Products

The strength and focus of BioConcept Ltd. lies in manufacturing customized cell culture media as well as defined media for recombinant protein production.

Besides that BioConcept Ltd. offers all standard (classical) media and solutions. Furthermore, the production facilities are superbly equipped to manufacture sterile QC liquids, microbial broths and agars. Therefore, our product line includes:

- Special customer-designed media
- Contract manufacturing of sterile liquids and powder formulations
- Production media for CHO, Hybridoma and Insect Cells
- Individual solutions for your cell culture requirements
- Complete cell systems applications for CHO cells
- Standard media
- Serum-free and ACF media
- Liquid as well as powder media formulations
- Buffers and balanced salt solutions
- Supplements and auxiliary reagents
- Animal sera

In addition to the broad range of cell culture products, we can offer the highest degree of flexibility and customization in a timely manner. Our customers have the following options:

- Modifications of standard products
- New products according to customers recipes
- Outsourcing of media production
- Variable batch sizes starting from 5 L up to 5 000 L (liquid) and 2 kg up to 800 kg (powder)
- Variable packaging sizes (1 ml up to 1 000 L) and packaging systems (PET/glass bottles, sterile bags, customized tubing systems, as well as customer specifications)
- Sterilization through sterile filtration (0.22 µm) or hot air/vapour sterilization

Customized products can be delivered within six to eight weeks after the order has been placed, including QC. For more detailed information please contact us at info@bioconcept.ch.



Classical Media

Basal Medium Eagle with EBS
Dulbecco's MEM (DMEM)
Dulbecco's MEM Low Glucose
Dulbecco's MEM High Glucose
DMEM/Ham's F-12 (1:1)
Ham's F-10 Medium
Ham's F-12 Medium
IMDM
Leibovitz L-15 Medium
Medium 199
McCoy's Medium
MEM Alpha
MEM EBS
MEM HBS
RPMI 1640
Waymouth's Medium
William's E Medium

Manta Ray [*Manta birostris*]

Manta Rays, also known as devil fish due to their devilish horns, can reach the massive width of 7 meters. Because of their size, and general appearance, they were often feared by sailors who believed they could sink their boats. In 1978, however, divers in California found the animal to be surprisingly friendly. While they normally live alone, large groups have been seen together during times of migration and at points called "cleaning stations", where the mantas are cleaned of parasites by small fish looking for an easy snack.



Classical Media

Basal Medium Eagle with EBS

Basal Medium Eagle (BME) was originally developed for studies to determine essential nutrient requirements of mouse "L" cells and HeLa cells in culture. This medium with a host of modifications is widely used and supports both the primary culture of a wide variety of normal mammalian and transformed cell lines. BioConcept Ltd. offers BME liquid with either Earl's Standard formula, Hank's salts and/or stable Glutamine.

Basal Medium Eagle

Cat. No.	1-06F01-I BME EBS
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Concentration		Amino Acids	
	mg/L		mg/L
Inorganic Salts		Amino Acids	
CaCl ₂ 2H ₂ O	265	L-Phenylalanine	16.5
KCl	400	L-Threonine	24
MgSO ₄ 7H ₂ O	200	L-Tryptophan	4
NaH ₂ PO ₄ H ₂ O	140	L-Tyrosine	18
NaCl	6800	L-Valine	23.5
NaHCO ₃	2220	Vitamins	
Other Components		myo-Inositol	1
D(+)-Glucose	1 000	(+)-Biotin	1
Phenol Red	10	D-Pantothenic acid Calcium salt	1
Amino Acids		Choline chloride	1
L-Arginine HCl	21	Folic acid	1
L-Cystine	12	Nicotinamide	1
L-Histidine HCl H ₂ O	10.81	Pyridoxal HCl	1
L-Isoleucine	26	Vitamin B2	0.1
L-Leucine	26	Vitamin B1 HCl	1
L-Lysine HCl	36.5	To be added separately	
L-Methionine	7.5	L-Glutamine	292

Available Basal Medium Eagle

Cat. No	Modification	Size
1-06F01-I	BME with EBS w/o L-Gln	500 ml
1-06F50-I	BME with EBS, with stable Gln (434 mg/L)	500 ml
1-08F01-I	BME with HBSS, w/o l-Gln	500 ml
1-08F50-I	BME with HBSS, with stable Gln (434 mg/L)	500 ml

Other modifications are available upon request at info@bioconcept.ch.

References

1. Eagle, H. (1955) Proc. Soc. Exp. Biol. Med 89, 362
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3. Eagle, W.R. (1956) Ann. NY. Acad. Sci. 63, 666
4. Eagle, H. (1959) Science 130, 432

Dulbecco's MEM (DMEM)

Dulbecco's MEM is the most widely used modification of BME. It contains four times the concentration of amino acids and vitamins. Non-essential amino acids and certain essential trace elements were added and the bicarbonate concentration was increased. The standard formula is with 1 000 mg/L glucose, the "high glucose" variation with 4 500 mg/L. Dulbecco's MEM was originally developed for the culture of mouse embryonic cells. Today it finds a broad application for serum free culture of normal and transformed mouse and chicken cells, e.g. 1:1 with Ham's F-12.

Dulbecco's MEM Low Glucose

Cat. No.	1-25F01-I DMEM Low Glucose
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Concentration		mg/L	
Inorganic Salts		Amino Acids	
CaCl ₂ 2H ₂ O	264	L-Methionine	30
Fe(NO ₃) ₃ 9H ₂ O	0.1	L-Phenylalanine	66
MgSO ₄ 7H ₂ O	200	L-Serine	42
KCl	400	L-Threonine	95
NaCl	6400	L-Tryptophan	16
NaH ₂ PO ₄ H ₂ O	125	L-Tyrosine	72
NaHCO ₃	3700	L-Valine	94
Other Components		Vitamins	
D(+)-Glucose	1000	Choline chloride	4
Phenol Red	15	D-Pantothenic acid calcium salt	4
Pyruvic acid sodium salt	110	Folic acid	4
Amino Acids		myo-Inositol	7.2
Glycine	30	Nicotinamide	4
L-Arginine HCl	84	Vitamin B1 HCl	4
L-Cystine	48	Vitamin B2	0.4
L-Histidine HCl H ₂ O	42	Vitamin B6 HCl	4
L-Isoleucine	105	To be added separately	
L-Leucine	105	L-Glutamine	584
L-Lysine HCl	146		

Available Dulbecco's MEM Low Glucose Media

Cat. No	Modification	Size
1-25F01-I	DMEM LG w/o L-Gln	500ml
1-25F03-I	DMEM LG with L-Gln	500ml
1-25F04-I	DMEM LG w/o L-Gln, with 25 mM HEPES	500ml
1-25F22-I	DMEM LG w/o L-Gln w/o Phenol Red	500ml
1-25F23-I	DMEM LG (1.0 g/L) with stable Gln (868 mg/L), w/o Phenol Red	500ml
1-25F24-I	DMEM LG w/o L-Gln, with 25 mM HEPES, w/o Phenol Red	500ml
1-25F50-I	DMEM LG with stable Gln (868 mg/L)	500ml
1-25F51-I	DMEM LG with 25 mM HEPES, with stable Gln (868 mg/L)	500ml
1-25K03-I	DMEM LG 10 × w/o L-Gln, w/o NaHCO ₃ , w/o Folic Acid	500ml
1-25K34-I	DMEM LG 10 × w/o L-Gln, w/o NaHCO ₃ , w/o Folic Acid, w/o Phenol Red	500 ml
1-25S16-I	DMEM w/o L-Gln, w/o Glucose	500ml
1-25P02-K, -L, -M, -N, -W	DMEM LG Powder, with L-Gln	1 L, 5 L, 10 L, 50 L, 100 L
1-25P32-K, -L, -M, -N, -W	DMEM LG Powder, with L-Gln, w/o Phenol Red	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

Dulbecco's MEM High Glucose

Cat. No.	1-26F01-I DMEM High Glucose
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Concentration

Inorganic Salts	mg/L	Amino Acids	mg/L
CaCl ₂ 2H ₂ O	264	L-Methionine	30
Fe(NO ₃) ₃ 9H ₂ O	0.1	L-Phenylalanine	66
MgSO ₄ 7H ₂ O	200	L-Serine	42
KCl	400	L-Threonine	95
NaCl	6400	L-Tryptophan	16
NaH ₂ PO ₄ H ₂ O	125	L-Tyrosine	72
NaHCO ₃	3700	L-Valine	94
Other Components		Vitamins	
D(+)-Glucose	4500	Choline chloride	4
Phenol Red	15	D-Pantothenic acid calcium salt	4
Amino Acids		Folic acid	4
Glycine	30	myo-Inositol	7.2
L-Arginine HCl	84	Nicotinamide	4
L-Cystine	48	Vitamin B1 HCl	4
L-Histidine HCl H ₂ O	42	Vitamin B2	0.4
L-Isoleucine	105	Vitamin B6 HCl	4
L-Leucine	105	To be added separately	
L-Lysine HCl	146	L-Glutamine	584

Available Dulbecco's MEM High Glucose Media

Cat. No	Modification	Size
1-26F01-I	DMEM HG w/o L-Gln	500ml
1-26F03-I	DMEM HG with L-Gln	500ml
1-26F04-I	DMEM HG w/o L-Gln, with 25 mM HEPES	500ml
1-26F22-I	DMEM HG w/o L-Gln w/o Phenol Red	500ml
1-26F23-I	DMEM HG (4.5 g/L) with stable Gln (868 mg/L), w/o Phenol Red	500ml
1-26F50-I	DMEM HG w/o L-Gln, with stable Gln (868 mg/L)	500ml
1-26F51-I	DMEM HG with stable Gln (868 mg/L), with 25 mM HEPES	500ml
1-26F55-I	DMEM HG with 25 mM HEPES, with Sodiumpyruvate (110 mg/L)	500ml
1-26F58-I	DMEM High Glucose with Sodiumpyruvate and L-Glutamine	500ml
1-26S31-I	DMEM HG w/o L-Gln, w/o L-Arg, w/o L-Lys	500ml
1-26P02-K, -L, -M, -N, -W	DMEM HG Powder, with L-Gln	1 L, 5 L, 10 L, 50 L, 100 L
1-26P32-K, -L, -M, -N, -W	DMEM HG Powder, with L-Gln, w/o Phenol Red	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

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DMEM / Ham's F-12 (1:1)

Cat. No.	1-26F08-I DMEM/F12		
Concentration			
Inorganic Salts	mg/L	Amino Acids	
		mg/L	
CaCl ₂ 2H ₂ O	154	L-Cystine	24
CuSO ₄ 5H ₂ O	0.00125	L-Glutamic acid	7.35
Na ₂ HPO ₄ anhydrous	71	L-Histidine HCl H ₂ O	31.48
Fe(NO ₃) ₃ 9H ₂ O	0.05	L-Isoleucine	54.45
FeSO ₄ 7H ₂ O	0.417	L-Leucine	59.05
MgCl ₂ 6H ₂ O	61	L-Lysine HCl	91.25
MgSO ₄ 7H ₂ O	100	L-Methionine	17.24
KCl	311.8	L-Phenylalanine	35.48
NaCl	6999.5	L-Proline	17.25
NaH ₂ PO ₄ H ₂ O	62.5	L-Serine	26.25
NaHCO ₃	2438	L-Threonine	53.45
ZnSO ₄ 7H ₂ O	0.43	L-Tryptophan	9.02
Other Components		L-Tyrosine	38.7
1,4-Diaminobutane 2 HCl	0.08	L-Valine	52.85
D(+)-Glucose	3151	Vitamins	
DL-alpha-Lipoic acid	0.105	(+)-Biotin	0.00365
Hypoxanthine	2.05	Choline chloride	8.98
Linoleic acid	0.042	D-Pantothenic acid calcium salt	2.24
Phenol Red	8.1	Folic acid	2.65
Pyruvic acid sodium salt	55	myo-Inositol	12.6
Thymidine	0.365	Nicotinamide	2.02
Amino Acids		Vitamin B1 HCl	2.17
Glycine	18.75	Vitamin B2	0.219
L-Alanine	4.45	Vitamin B6 HCl	2.031
L-Arginine HCl	147.5	Vitamin B12	0.68
L-Asparagine H ₂ O	7.5	To be added separately	
L-Aspartic acid	6.65	L-Glutamine	365
L-Cysteine HCl H ₂ O	17.56		

Available DMEM / Ham's F-12 (1:1) Media

Cat. No	Modification	Size
1-26F07-I	DMEM/F-12 w/o L-Gln, with 15 mM HEPES	500ml
1-26F08-I	DMEM/F-12 w/o L-Gln	500ml
1-26F09-I	DMEM/F-12 with stable Gln (543 mg/L)	500ml
1-57F22-I	DMEM/F-12 w/o L-Gln, w/o Phenol Red	500ml
1-57F23-I	DMEM/F-12 with stable Gln (543 mg/L), w/o Phenol Red	500ml
1-57F24-I	DMEM/F-12 w/o L-Gln, with 15 mM HEPES w/o Phenol Red	500ml
1-57F51-I	DMEM/F-12 with 15 mM HEPES, with stable Gln (543 mg/L)	500ml
1-57F54-I	DMEM/F-12 with 15 mM HEPES, with stable Gln (543 mg/L), w/o Phenol Red	500ml
1-57P02-K, -L, -M, -N, -W	DMEM/F-12 Powder with L-Gln	1 L, 5 L, 10 L, 50 L, 100 L
1-57P06-K, -L, -M, -N, -W	DMEM/F-12 Powder with L-Gln, with 15 mM HEPES, w/o Phenol Red	1 L, 5 L, 10 L, 50 L, 100 L
1-57P32-K, -L, -M, -N, -W	DMEM/F-12 Powder with L-Gln, w/o Phenol Red	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

Ham's F-10 Medium

Ham's F-10 Medium is a complex formula containing amino acids, vitamins and certain supplements and ingredients at optimum concentrations. It was originally developed for serum free culture of Chinese Hamster Ovary (CHO) cells, HeLa cell clones and mouse "L" cells. Ham's F-10 is today a widely established and popular medium for the growth of fastidious cell lines with serum or serum free, eg. diploid human cells, white blood cells for chromosomal analysis, primary explants of rat, chicken and rabbit.

Cat. No.		1-13F01-I Ham's F-10	
Concentration			
Inorganic Salts		mg/L	Amino Acids
			mg/L
CaCl ₂ 2H ₂ O	44	L-Histidine HCl H ₂ O	23
CuSO ₄ 5H ₂ O	0.0025	L-Isoleucine	2.6
Na ₂ HPO ₄ anhydrous	154	L-Leucine	13.1
FeSO ₄ 7H ₂ O	0.834	L-Lysine HCl	29
MgSO ₄ 7H ₂ O	153	L-Methionine	4.48
KCl	285	L-Phenylalanine	4.96
KH ₂ PO ₄	83	L-Proline	11.5
NaCl	7400	L-Serine	10.5
NaHCO ₃	1200	L-Threonine	3.6
ZnSO ₄ 7H ₂ O	0.0283	Tryptophan	0.6
Other Components		L-Tyrosine	1.81
D(+)-Glucose	1100	L-Valine	3.5
DL-alpha-Lipoic acid	0.21	Vitamins	
Hypoxanthine	4.1	(+)-Biotin	0.024
Phenol Red	1.2	Choline chloride	0.7
Pyruvic acid sodium salt	110	D-Pantothenic acid Calcium salt	0.715
Thymidine	0.7	Folic acid	1.32
Amino Acids		myo-Inositol	0.542
Glycine	7.5	Nicotinamide	0.615
L-Alanine	9	Vitamin B1 HCl	1
L-Arginine HCl	211	Vitamin B2	0.375
L-Asparagine H ₂ O	15	Vitamin B6 HCl	0.205
L-Aspartic acid	13.3	Vitamin B12	1.36
L-Cysteine	25	To be added separately	
L-Glutamic acid	14.7	L-Glutamine	146

Available Ham's F-10 Media

Cat. No	Modification	Size
1-13F01-I	Ham's F-10 w/o L-Gln	500 ml
1-13F02-I	Ham's F-10 with L-Gln	500 ml
1-13F04-I	Ham's F-10 w/o L-Gln, with 25 mM HEPES	500 ml
1-13F22-I	Ham's F-10 w/o L-Gln, w/o Phenol Red	500 ml
1-13F50-I	Ham's F-10 with stable Gln (217 mg/L)	500 ml

Other modifications are available upon request at info@bioconcept.ch.

References

- Ham, R.G. (1963) Exp. Cell Res. 29, 515-526
- Ham, R.G. (1965) Proc. Nat. Acad. Sci. 53, 288-293

Ham's F-12 Medium

Ham's F-12 Medium is a modification of the complex Ham's F-10 formula, containing amino acids, vitamins and trace elements at optimum concentrations. Originally it was developed for serum free culture of Chinese Hamster Ovary (CHO) cells, primary Rat Hepatocytes and Prostate Epithelial Cells. Ham's F-12 is today the medium of choice for the clonal toxicity assay with CHO-cells (CHD-3 and CHL-1) and supports the growth of a variety of normal, fastidious and transformed cells with serum or serum free.

Cat. No.	1-14F01-I Ham's F-12
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Concentration		Amino Acids	
Inorganic Salts	mg/L		mg/L
CaCl ₂ 2H ₂ O	44	L-Histidine HCl H ₂ O	20.9
CuSO ₄ 5H ₂ O	0.0025	L-Isoleucine	3.9
Na ₂ HPO ₄ anhydrous	142	L-Leucine	13.1
FeSO ₄ 7H ₂ O	0.834	L-Lysine HCl	36.5
MgCl ₂ 6H ₂ O	122	L-Methionine	4.48
KCl	223.6	L-Phenylalanine	4.96
NaCl	7599	L-Proline	34.5
NaHCO ₃	1176	L-Serine	10.5
ZnSO ₄ 7H ₂ O	0.86	L-Threonine	11.9
Other Components		L-Tryptophan	2.04
2'-Deoxythymidine	0.73	L-Tyrosine	5.4
1,4-Diaminobutane 2 HCl	0.16	L-Valine	11.7
D(+)-Glucose	1802	Vitamins	
DL-alpha-Lipoic acid	0.21	(+)-Biotin	0.0073
Hypoxanthine	4.1	Choline chloride	13.96
Linoleic acid	0.084	D-Pantothenic acid calcium salt	0.48
Phenol Red	1.2	Folic acid	1.32
Pyruvic acid Sodium salt	110	myo-Inositol	18
Amino Acids		Nicotinamide	0.037
Glycine	7.5	Vitamin B1 HCl	0.34
L-Alanine	9	Vitamin B12	1.36
L-Arginine HCl	211	Vitamin B2	0.038
L-Asparagine H ₂ O	15	Vitamin B6 HCl	0.062
L-Aspartic acid	13.3	To be added separately	
L-Cysteine HCl H ₂ O	35.12	L-Glutamine	146
L-Glutamic acid	14.7		

Available Ham's F-12 Media

Cat. No	Modification	Size
1-14F01-I	Ham's F-12 w/o L-Gln	500 ml
1-14F03-I	Ham's F-12 with L-Gln	500 ml
1-14F04-I	Ham's F-12 w/o L-Gln, with 25 mM HEPES	500 ml
1-14F22-I	Ham's F-12 w/o L-Gln, w/o Phenol Red	500 ml
1-14F50-I	Ham's F-12 with stable Gln (217 mg/L)	500 ml
1-14K03-I	Ham's F-12 10 x, w/o L-Gln, w/o NaHCO ₃	500 ml
1-14K34-I	Ham's F-12 10 x, w/o L-Gln, w/o Phenol Red, w/o NaHCO ₃	500 ml
1-14P02-K, -L, -M, -N, -W	Ham's F-12 Powder with L-Gln	1 L, 5 L, 10 L, 50 L, 100 L
1-14S50-I	Ham's F-12 Kaighn's Modification with stable Gln (434 mg/L)	500 ml

Other modifications are available upon request at info@bioconcept.ch.

References

1. Ham, R.G.; (1963) Exp.Cell.Res. 29, 515
2. Ham, R.G.; (1965) Proc.Nat.Acad.Sci. 53, 288
3. Barnes, D. et al.; (1980) Cell. 22, 649
4. Dulbecco, R. et al.; (1959) Virology, 8 396
5. Smith, J.D. et al.; (1960) Virology 12, 185
6. Morton, H.J.; (1970) In Vitro 6, 89
7. Rutzky, L.P. et al.; (1974) in Vitro 9, 468
8. Ham, R.G. et al.; (1979) Meth. Enzymol. 53, 44
9. Barnes, D. et al.; (1980) Anal.Biochem. 102, 255
10. Kaighn, M.E.; TCMA (1973) 54-58

Iscove's IMDM

Iscove's IMDM, is the most known and widely used modification of Dulbecco's MEM. It is a completely defined, serum free medium, supplemented with albumin, transferrin and lecithin, selenium, amino acids, vitamins and HEPES buffer, pyruvate and KNO₃ instead of Fe(NO₃)₃. Iscove's IMDM was originally developed for rapid propagation of erythropoietic and hemopoietic bone marrow precursor cells in serum free media. It is ideal also for hybridoma and T-/B-Lymphocytes cell culture of both, human and mouse.

Cat. No.	1-28F16-I IMDM w/o Supplements
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Concentration		mg/L	
Inorganic Salts			
CaCl ₂ 2H ₂ O		219	
MgSO ₄ 7H ₂ O		200	
KCl		330	
KNO ₃		0.076	
NaCl		4505	
NaH ₂ PO ₄ 2H ₂ O		141	
NaHCO ₃		3024	
NaSeO ₃ 5H ₂ O		0.017	
Other Components			
D(+)-Glucose		4500	
HEPES		5958	
Phenol Red		15	
Pyruvic acid sodium salt		110	
Amino Acids			
Glycine		30	
L-Alanine		25	
L-Arginine HCl		84	
L-Asparagine H ₂ O		28.4	
L-Aspartic acid		30	
L-Cystine		70	
L-Glutamic acid		75	
L-Histidine HCl H ₂ O		42	
L-Isoleucine		105	
L-Leucine		105	
L-Lysine HCl		146	
L-Methionine		30	
Amino Acids			
L-Phenylalanine		66	
L-Proline		40	
L-Serine		42	
L-Threonine		95	
L-Tryptophan		16	
L-Tyrosine		72	
L-Valine		94	
Vitamins			
(+)-Biotin		0.013	
Choline chloride		4	
D-Pantothenic acid calcium salt		4	
Folic acid		4	
myo-Inositol		7.2	
Nicotinamide		4	
Vitamin B1 HCl		4	
Vitamin B2		0.4	
Vitamin B6 HCl		4	
Vitamin B12		0.013	
To be added separately			
L-Glutamine		584	
Iscove's Supplemented Media contain			
BSA		400	
Linoleic acid		1	
Oleic acid		1	
Palmitic acid		1	
Transferrin		1	

Available Iscove's IMDM Media

Cat. No	Modification	Size
1-28F01-I	IMDM w/o L-Gln, with Supplements	500 ml
1-28F02-I	IMDM with L-Gln, with Supplements	500 ml
1-28F16-I	IMDM w/o L-Gln, w/o Supplements	500 ml
1-28F17-I	IMDM with L-Gln, w/o Supplements	500 ml
1-28F22-I	IMDM w/o L-Gln, w/o Supplements, w/o Phenol Red	500 ml
1-28F50-I	IMDM w/o Supplements, with stable Gln (868 mg/L)	500 ml
1-28P17-K, -L, -M, -N, -W	IMDM Powder, with L-Gln, w/o Supplements	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

References

1. Iscove, N.N. et. al.; (1978) J.Exp.Med. 147, 923
2. Iscove, N.N. et. al.; (1980) Exp.Cell.Res. 126, 121
3. Dulbecco, R. et al.; (1959) Virology, 8, 396
4. Harbour, C. et. al.; (1991) in Mammalian Cell Biotechnology, 1st Ed.; IRL Press, Oxford – NY – Tokyo

Leibovitz L-15 Medium

Leibovitz L-15 Medium is a complex NaHCO_3 -free formulation for use in carbon dioxide free cell culture systems. It is buffered by its complement salts, free base amino acids, galactose and phosphates to maintain physiological pH conditions. Leibovitz L-15 Medium supports the growth and proliferation of a variety of established cell lines, eg. HEp-2, as well as primary explants of embryonic and adult human tissues, eg. nerve tissue. It is supplemented with 10% Tryptose Phosphate Broth, L-15 is highly suitable for the culture of insect cells, e.g. bed bug, mosquitoes and ticks.

Cat. No.	1-17F01-I Leibovitz L-15
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Concentration		Concentration	
Inorganic Salts	mg/L	Amino Acids	mg/L
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	185,4	L-Asparagine anhydrous	250
Na_2HPO_4 anhydrous	190	L-Cysteine	120
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	200	L-Histidine	250
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	200	L-Leucine	125
KCl	400	L-Lysine	75
KH_2PO_4	60	L-Serine	200
NaCl	8000	L-Tryptophan	20
Other Components		L-Tyrosine	300
D(+)-Galactose	900	Vitamins	
Phenol Red	10	Choline chloride	1
Pyruvic acid sodium salt	550	D-Pantothenic acid calcium salt	1
Amino Acids		Folic acid	1
L-Alanine	225	myo-Inositol	2
L-Isoleucine	125	Nicotinamide	1
L-Methionine	75	Riboflavin 5-Phosphate Na	0.1
L-Phenylalanine	125	Thiamin monophosphate	1
L-Threonine	300	Vitamin B6 HCl	1
L-Valine	100	To be added separately	
Glycine	200	L-Glutamine	300
L-Arginine	500		

Other modifications are available upon request at info@bioconcept.ch.

References

1. Leibovitz, A.; (1963) Amer.J.Hyg. 78, 173
2. Yunker, C.E. et al.; (1979) TCA Manual 5, 1077

Medium 199

Medium 199 is a totally defined nutritive source for explant tissues and cells in cell culture. Properly supplemented with serum, Medium 199 offers broad applicability for cells of different animal species, particularly for the culture of non-transformed cells. It is also used in virology for vaccine production.

Cat. No.	1-21F01-I M 199 EBS
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Concentration		Concentration	
Inorganic Salts	mg/L	Amino Acids	mg/L
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	265	L-Glutamic acid	66.82
$\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$	0.72	L-Histidine HCL, H_2O	21.88
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	200	L-Isoleucine	20
KCl	400	L-Leucine	60
NaCl	6800	L-Lysine HCl	70
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	140	L-Methionine	15
NaHCO_3	2200	L-Phenylalanine	25
Other Components		L-Proline	40
2-Deoxy-D-ribose	0.5	L-Serine	25
4-Aminobenzoic acid	0.05	L-Threonine	30
Adenine sulfate	10	L-Tryptophan	10
AMP H_2O	0.25	L-Tyrosine	40
ATP disodium salt H_2O	1	L-Valine	25
Cholesterol	0.2	Vitamins	
D(+)-Glucose	1000	(+)-Biotin	0.01
D(-)-Ribose	0.5	Vitamin K3	0.02
Guanine HCl	0.3	Choline chloride	0.5
Hypoxanthine	0.3	D-Pantothenic acid calcium salt	0.01
L-Glutathione reduced	0.05	Folic acid	0.01
Phenol Red	20	myo-Inositol	0.05
CH_3COONa anhydrous	50	Nicotinamide	0.025
Thymine	0.3	Nicotinic acid	0.025
Tween 80	20	Pyridoxal HCl	0.025
Uracil	0.3	Vitamin B1 HCl	0.01
Xanthine	0.3	Vitamin B2	0.01
Amino Acids		Vitamin B6 HCl	0.025
Glycine	50	Vitamin C	0.05
L-4-Hydroxyproline	10	Vitamin D2	0.1
L-Alanine	25	Vitamin E succinate	0.01
L-Arginine HCl	70	Vitamin A acetate	0.14
L-Aspartic acid	30	To be added separately	
L-Cysteine HCL, H_2O	0.11	L-Glutamine	100
L-Cystine	20		

Cat. No.	1-22F01-I M 199 HBS (Liquid)
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Concentration

Amino Acids	mg/L	Vitamins	mg/L
L-Alanine	25	p-Aminobenzoic Acid	0.05
L-Arginine, HCl	70	Pyridoxin, HCl	0.025
L-Aspartic Acid	30	Pyridoxal, HCl	0.025
L-Cysteine, HCl H ₂ O	0.11	Riboflavin	0.01
L-Cystine	20	Thiamine, HCl	0.01
L-Glutamic Acid	66.82	Vitamin A-acetate	0.14
Glycin	50	Inorganic Salts	
L-Histidine, HCl H ₂ O	21.88	CaCl ₂ 2H ₂ O	185.5
L-4-Hydroxyproline	10	NaCl	8000
L-Isoleucine	20	KCl	400
L-Leucine	60	Fe(NO ₃) ₃ 9H ₂ O	0.72
L-Lysine, HCl	70	KH ₂ PO ₄	60
L-Methionine	15	MgSO ₄ 7H ₂ O	200
L-Phenylalanine	25	NaH ₂ PO ₄ anhydrous	47.5
L-Proline	40	NaHCO ₃	350
L-Serine	25	Others	mg/L
L-Threonine	30	Adenosine 5'-triphosphate 2Na H ₂ O	1
L-Tryptophan	10	Adenosine 5'-phosphate H ₂ O	0.25
L-Tyrosin	40	Adenine Sulphate	10
L-Valine	25	L-Glutathione (red.)	0.05
Vitamins		Guanine HCl	0.3
Ascorbic Acid	0.05	Deoxy-D-ribose	0.5
Tocopherolsuccinate DL-alfa	0.01	D-Ribose	0.5
D-Biotin	0.01	Glucose, D (+)	1000
Calciferol	0.1	Hypoxanthine	0.3
D-Pantothenic Acid (Ca-Salt)	0.01	Sodium Acetate (anhydr.)	50
Choline Chloride	0.5	Tween 80	20
Folic Acid	0.01	Xanthine	0.3
Inositol	0.05	Uracil	0.3
Menadione	0.02	Thymine	0.3
Niacin	0.025	Phenol red	20
Niacinamide	0.025	Cholesterol	0.2

Available Medium 199 Media

Cat. No	Modification	Size
1-21F01-I	M 199 EBS w/o L-Gln	500 ml
1-21F04-I	M 199 EBS w/o L-Gln, with 25 mM HEPES	500 ml
1-21F22-I	M 199 EBS w/o L-Gln w/o Phenol Red	500 ml
1-21F50-I	M 199 EBS with stable Gln (149 mg/L)	500 ml
1-22F01-I	M 199 HBS w/o L-Gln	500 ml
1-22F50-I	M 199 HBS with stable Gln (149 mg/L)	500 ml

Other modifications are available upon request at info@bioconcept.ch.

References

1. Morgan, J.F. et al.; (1950) Proc.Soc.Exp.Biol.Med. 73, 1
2. Morgan, J.F. et al.; (1951) Nat. Canc.Inst. 16/2, 557

McCoy's 5A

McCoy's 5A Medium was created using Basal Medium 5A and is a nutritive substance rich complete medium. McCoy's 5A Medium was originally developed to investigate the amino acid requirements of Novikoff Hepatoma cells. Today this optimal developed medium has a broad application to support the growth of the most demanding primary and continuous cell lines, as well as from biopsy tissues, eg. bone marrow, skin, kidney, omentum, adrenal glands, lung, spleen.

Cat. No.	1-18F01-I McCoy's 5A
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Concentration

Inorganic Salts	mg/L	Amino Acids	mg/L
CaCl ₂ 2H ₂ O	132.5	L-Methionine	14.9
MgSO ₄ 7H ₂ O	200	L-Phenylalanine	16.5
KCl	400	L-Proline	17.3
NaCl	6460	L-Serine	26.3
NaH ₂ PO ₄ H ₂ O	580	L-Threonine	17.9
NaHCO ₃	2200	L-Tryptophan	3.1
Other Components		L-Tyrosine	18.1
4-Aminobenzoic acid	1	L-Valine	17.6
Bacto Peptone	600	Vitamins	
D(+)-Glucose	3000	(+)-Biotin	0.2
L-Glutathione reduced	0.5	Choline chloride	5
Phenol Red	10	D-Pantothenic acid calcium salt	0.2
Amino Acids		Folic acid	10
Glycine	7.5	myo-Inositol	36
L-4-Hydroxyproline	19.7	Nicotinamide	0.5
L-Alanine	13.9	Nicotinic acid	0.5
L-Arginine HCl	42.1	Pyridoxal HCl	0.5
L-Asparagine anhydrous	45	Vitamin B1 HCl	0.2
L-Aspartic acid	19.97	Vitamin B2	0.2
L-Cysteine	31.5	Vitamin B6 HCl	0.5
L-Glutamic acid	22.1	Vitamin B12	2
L-Histidine HCl H ₂ O	20.96	Vitamin C	0.5
L-Isoleucine	39.36	To be added separately	
L-Leucine	39.36	L-Glutamine	219.2
L-Lysine HCl	36.5		

Available McCoy's 5A Media

Cat. No	Modification	Size
1-18F01-I	McCoy's 5A w/o L-Gln	500 ml
1-18F03-I	McCoy's 5A with L-Gln	500 ml
1-18F22-I	McCoy's 5A w/o L-Gln (326 mg/L), w/o Phenol Red	500 ml
1-18F23-I	McCoy's 5A with stable Gln (326 mg/L), w/o Phenol Red	500 ml
1-18F50-I	McCoy's 5A with stable Gln (326 mg/L)	500 ml
1-18F51-I	McCoy's 5A with 25 mM HEPES, with stable Gln (326 mg/L)	500 ml
1-18P02-K, -L, -M, -N, -W	McCoy's 5A Powder, with L-Gln	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

References

1. Neumann, R.E. et al.; (1958) Proc.Soc.Exp.Biol.Med. 98, 303
2. McCoy, T.A. et al.; (1959) Proc.Soc.Exp.Biol.Med. 100, 115
3. Hsu, T.C. et al.; (1960) J.Natl.Canc.Inst. 25, 221
4. Iwakata, S. et al.; (1964) N.Y. State J.Med. 64, 2279
5. Morton, H.J.; (1970) In Vitro 6, 89
6. Park, M.S. et al.; (1974) Transpl. 18, 520
7. Patterson, M.K. et al.; (1978) TCA Manual 4, 737

MEM Alpha

MEM Alpha is a highly complex formulation and is enriched with additional amino acids, vitamins, lipoic acid and pyruvate. It is highly enriched if supplemented with ribo- and desoxyribonucleosides. Originally used for growth of hamster kidney cells, MEM Alpha supports the growth and proliferation of bone marrow and amniotic cells, both in monolayer- and suspension-culture. Supplementation with serum or certain proteins supports the culture. BioConcept Ltd. offers MEM Alpha with and without nucleosides as liquid/powder variations. Modifications are possible on customer requests.

Cat. No.	1-23F01-I MEM Alpha with Nucleosides
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Concentration		Concentration	
	mg/L		mg/L
Inorganic Salts			
CaCl ₂ 2H ₂ O	265	Amino Acids	
MgSO ₄ 7H ₂ O	200	L-Threonine	48
KCl	400	L-Tryptophan	10
NaCl	6800	L-Tyrosine	36
NaH ₂ PO ₄ H ₂ O	140	L-Valine	46
NaHCO ₃	2200	Vitamins	
Other Components			
D(+)-Glucose	1000	(+)-Biotin	0.1
DL-alpha-lipoic acid	0.2	Choline chloride	1
Phenol Red	10	D-Pantothenic acid calcium salt	1
Pyruvic acid sodium salt	110	Folic acid	1
Amino Acids			
Glycine	50	myo-Inositol	2
L-Alanine	25	Nicotinamide	1
L-Arginine HCl	127	Pyridoxal HCl	1
L-Asparagine H ₂ O	50	Vitamin B1 HCl	1
L-Aspartic acid	30	Vitamin B2	0.1
L-Cysteine	100	Vitamin B12	1.4
L-Cystine	24	Vitamin C	50
L-Glutamic acid	75	Ribonucleosides	
L-Histidine HCl H ₂ O	42	Adenosine	10
L-Isoleucine	52	Cytidine	10
L-Leucine	52	Guanosine	10
L-Lysine HCl	73	Uridine	10
L-Methionine	15	Deoxyribonucleosides	
L-Phenylalanine	32	2'-Deoxyadenosine	10
L-Proline	40	2'-Deoxycytidine HCl	11
L-Serine	25	2'-Deoxyguanosine H ₂ O	10.67
		2'-Deoxythymidine	10
		To be added separately	
		L-Glutamine	292

Available MEM Alpha Media

Cat. No	Modification	Size
1-23F01-I	MEM Alpha w/o L-Gln, with Nucleosides	500 ml
1-23F09-I	MEM Alpha w/o L-Gln, w/o Nucleosides	500 ml
1-23F22-I	MEM Alpha w/o L-Gln, w/o Nucleosides, w/o Phenol Red	500 ml
1-23F23-I	MEM Alpha w/o L-Gln, with Nucleosides, w/o Phenol Red	500 ml
1-23S50-I	MEM Alpha with stable Gln (434 mg/L), with Nucleosides	500 ml
1-23F50-I	MEM Alpha with stable Gln (434 mg/L), w/o Nucleosides	500 ml
1-23P10-K, -L, -M, -N, -W	MEM Alpha w/o Nucleosides, Powder	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

References

1. Stanners, C.P. et al.; (1971) Nat.New.Biol. 230, 52
2. Stanners, C.P. et al.; (1975) J.Gen.Virol. 29, 281
3. Earle, W. (1943) J.Natl.Cancer.Inst. 4, 165

MEM EBS and MEM HBS

MEM Eagle, a complex amino acid and vitamin rich medium, was developed from Eagle's Basal Medium (BME). Based on Eagle's Hank's BSS, MEM is one of the most widely used of all synthetic cell culture media. MEM Eagle with EBS or HBS supports the growth and proliferation of a variety of fastidious primary mammalian cells and established cell lines, both in monolayer- and suspension-culture. Supplementation with serum or certain proteins supports the culture.

Cat. No.	1-31F01-I MEM EBS
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Concentration			
Inorganic Salts	mg/L	Amino Acids	mg/L
CaCl ₂ 2H ₂ O	267	L-Phenylalanine	33
MgSO ₄ 7H ₂ O	200	L-Threonine	47.6
KCl	400	L-Tryptophan	10.2
NaCl	6800	L-Tyrosine	36.2
NaH ₂ PO ₄ H ₂ O	140	L-Valine	46
NaHCO ₃	2200	Vitamins	
Other Components		Choline chloride	1
D(+)-Glucose	1000	D-Pantothenic acid calcium salt	1
Phenol Red	10	Folic acid	1
Amino Acids		myo-Inositol	2
L-Arginine HCl	126.4	Nicotinamide	1
L-Cystine	24	Pyridoxal HCl	1
L-Histidine HCl H ₂ O	41.93	Vitamin B1 HCl	1
L-Isoleucine	52	Vitamin B2	0.1
L-Leucine	52	To be added separately	
L-Lysine HCl	73.06	L-Glutamine	292
L-Methionine	14.9		

Available MEM EBS Media

Cat. No	Modification	Size
1-31F01-I	MEM EBS w/o L-Gln	500 ml
1-31F03-I	MEM EBS with L-Gln	500 ml
1-31F04-I	MEM EBS w/o L-Gln, with 25 mM HEPES	500 ml
1-31F18-I	MEM EBS w/o L-Gln, w/o Leucine	500 ml
1-31F19-I	MEM EBS w/o L-Gln, w/o Phosphate	500 ml
1-31F20-I	MEM EBS w/o L-Gln, w/o Methionine	500 ml
1-31F21-I	MEM EBS w/o L-Gln, with 25 mM HEPES, w/o Folic acid	500 ml
1-31F22-I	MEM EBS w/o L-Gln, w/o Arginine	500 ml
1-31F24-I	MEM EBS w/o L-Gln, w/o Phenol Red	500 ml
1-31F50-I	MEM EBS with stable Gln (434 mg/L)	500 ml
1-31F51-I	MEM EBS with 25 mM HEPES, with stable Gln (434 mg/L)	500 ml
1-31P02-K, -L, -M, -N, -W	MEM EBS Powder with L-Gln	1 L, 5 L, 10 L, 50 L, 100 L
1-31P05-K, -L, -M, -N, -W	MEM EBS Powder, with L-Gln, with 25 mM HEPES	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

References

1. Eagle, H. et al.; (1956) J.Biol.Chem. 214, 845
2. Eagle, H. (1955) Science 122, 501
3. Eagle, H. (1959) Science 130, 432
4. Parker, R.C. (1961) In: Meth.Tis.Cult.; 3rd Ed. Harper NY
5. Eagle, H. (1976) TCA Manual 3, 517
6. Daniel, M.D. et al.; (1969) Proc.Soc.Exp.Biol.Med. 127, 3
7. Ham, R.G. et al.; (1979) Meth.Enzymol. 53, 44
8. Earle, W. (1943) Natl.Cancer.Inst. 4, 165

MEM HBS

Cat. No.	1-33F01-I MEM HBS
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Concentration		Amino Acids	
Inorganic Salts	mg/L		mg/L
CaCl ₂ 2H ₂ O	185.4	L-Methionine	14.9
Na ₂ HPO ₄ anhydrous	47.5	L-Phenylalanine	33
MgSO ₄ 7H ₂ O	200	L-Threonine	47.6
KCl	400	L-Tryptophan	10.2
KH ₂ PO ₄	60	L-Tyrosine	36.2
NaCl	8000	L-Valine	46
NaHCO ₃	350	Vitamins	
Other Components		Choline chloride	1
D(+)-Glucose	1 000	D-Pantothenic acid calcium salt	1
Phenol Red	10	Folic acid	1
Amino Acids		myo-Inositol	2
L-Arginine HCl	126.4	Nicotinamide	1
L-Cystine	24	Pyridoxal HCl	1
L-Histidine HCl H ₂ O	41.93	Vitamin B1 HCl	1
L-Isoleucine	52	Vitamin B2	0.1
L-Leucine	52	To be added separately	
L-Lysine HCl	73.06	L-Glutamine	292

Available MEM HBS Media

Cat. No	Modification	Size
1-33F01-I	MEM HBS w/o L-Gln	500 ml
1-33F50-I	MEM HBS with stable Gln (434 mg/L)	500 ml
1-33F51-I	MEM HBS with 25 mM HEPES, with stable Gln (434 mg/L)	500 ml

Other modifications are available upon request at info@bioconcept.ch.

References

- Hanks, J.H.; (1976) TCA Manual 3, 3
- Hanks, J.H. et al.; (1949) Proc.Soc.Exp.Biol.Med. 71, 19

RPMI 1640

RPMI 1640 was developed as a modification of McCoy's 5A medium and is based on RPMI 1630. RPMI 1640 uses a bi-carbonate buffering system and differs from most mammalian cell culture media in its typical pH 8 formulation. With or without supplemented serum or other defined proteins, RPMI 1640 represents today's most widely applied culture medium for short as well as long term cultivation of many cell types, especially human T/B-lymphocytes (e.g. 72 h PHA stimulation assay), bone marrow cells, hybridoma cells.

Cat. No.	1-41F01-I RPMI 1640
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Concentration		Amino Acids	
Inorganic Salts	mg/L		mg/L
Ca(NO ₃) ₂ 4H ₂ O	100	L-Lysine HCl	40
Na ₂ HPO ₄ anhydrous	800	L-Methionine	15
MgSO ₄ 7H ₂ O	100	L-Phenylalanine	15
KCl	400	L-Proline	20
NaCl	6000	L-Serine	30
NaHCO ₃	2000	L-Threonine	20
Other Components		L-Tryptophan	5
4-Aminobenzoic acid	1	L-Tyrosine	20
D(+)-Glucose	2 000	L-Valine	20
L-Glutathione reduced	1	Vitamins	
Phenol Red	5	(+)-Biotin	0.2
Amino Acids		Choline chloride	3
Glycine	10	D-Pantothenic acid calcium salt	0.25
L-4-Hydroxyproline	20	Folic acid	1
L-Arginine HCl	241.9	myo-Inositol	35
L-Asparagine anhydrous	50	Nicotinamide	1
L-Aspartic acid	20	Vitamin B1 HCl	1
L-Cystine	50	Vitamin B2	0.2
L-Glutamic acid	20	Vitamin B6 HCl	1
L-Histidine	15	Vitamin B12	0.005
L-Isoleucine	50	To be added separately	
L-Leucine	50	L-Glutamine	300

Available RPMI 1640 Media

Cat. No	Modification	Size
1-41F01-I	RPMI 1640 w/o L-Gln	500 ml
1-41F03-I	RPMI 1640 with L-Gln	500 ml
1-41F04-I	RPMI 1640 w/o L-Gln with 25 mM HEPES	500 ml
1-41F22-I	RPMI 1640 w/o L-Gln, w/o Phenol Red	500 ml
1-41F23-I	RPMI 1640 with stable Gln (446 mg/L), w/o Phenol Red	500 ml
1-41F24-I	RPMI 1640 w/o L-Gln, with 25 mM HEPES, w/o Phenol Red	500 ml
1-41F50-I	RPMI 1640 with stable Gln (446 mg/L)	500 ml
1-41F51-I	RPMI 1640 with 25 mM HEPES, with stable Gln (446 mg/L)	500 ml
1-41F54-I	RPMI 1640, with 25 mM HEPES, with stable Gln, w/o Phenol Red	500 ml
1-41S07-I	RPMI 1640, w/o L-Gln, w/o Glucose	500 ml
1-41S37-I	RPMI 1640 w/o L-Gln, w/o L-Arg, w/o L-Lys	500 ml
1-41K03-I	RPMI 1640 10 x w/o L-Gln, w/o NaHCO ₃	500 ml
1-41K34-I	RPMI 1640 10 x w/o L-Gln, w/o NaHCO ₃ , w/o Phenol Red	500 ml
1-41P02-K, -L, -M, -N, -W	RPMI 1640 Powder with L-Gln	1 L, 5 L, 10 L, 50 L, 100 L
1-41P05-K, -L, -M, -N, -W	RPMI 1640 Powder with L-Gln, with 25 mM HEPES	1 L, 5 L, 10 L, 50 L, 100 L
1-41P32-K, -L, -M, -N, -W	RPMI 1640 Powder with L-Gln, w/o Phenol Red	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

References

- Moore, G.E. et al.; (1967) JAMA 199, 519
- Moore, G.E. et al.; (1976) TCA Manual, 3, 503
- Moore, G.E. et al.; (1967) XXI.Ann.Symp.Fund.Canc.Res.Feb., 41
- Moore, G.E. et al.; (1968) NY.State J.Med. 68, 2054
- Ham, R.G. et al.; (1979) Meth.Enzymol. 53, 44

Waymouth's Medium

Waymouth's Medium was developed as a serum free, chemically defined synthetic medium for the culture of mouse cell line L929.

Cat. No.	1-46F01-I Waymouth's MB752/1
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Concentration		mg/L		
Inorganic Salts				
CaCl ₂ 2H ₂ O	120	Amino Acids	mg/L	
MgCl ₂ 6H ₂ O	240	L-Lysine HCl	240	
MgSO ₄ 7H ₂ O	200	L-Methionine	50	
KCl	150	L-Phenylalanine	50	
NaCl	6000	L-Proline	50	
Na ₂ HPO ₄ anhydrous	300	L-Threonine	75	
KH ₂ PO ₄	80	L-Tryptophan	40	
NaHCO ₃	2240	L-Tyrosine	40	
Other Components				
D(+)-Glucose	5000	L-Valine	65	
Glutathione, reduced	15	Vitamins		
Hypoxanthine	25	(+)-Biotin	0.02	
Phenol Red	10	Choline chloride	250	
Amino Acids				
Glycine	50	D-(Pantothenic acid calcium salt)	1	
L-Arginine HCl	75	Folic acid	0.5	
L-Aspartic acid	60	myo-Inositol	1	
L-Cysteine HCl H ₂ O	100.3	Nicotinamide	1	
L-Cystine	15	Pyridoxine HCl	1	
L-Glutamic acid	150	Vitamin B1 HCl	10	
L-Histidine HCl H ₂ O	164.1	Vitamin B2	1	
L-Isoleucine	25	Vitamin B12	0.20	
L-Leucine	50	Vitamin C	17.5	
To be added separately				
			L-Glutamine	350

Available Waymouth's Media

Cat. No	Modification	Size
1-46F01-I	Waymouth's MB 752/1 w/o L-Gln	500 ml
1-46F22-I	Waymouth's MB 752/1 w/o L-Gln, w/o Phenol Red	500 ml

Other modifications are available upon request at info@bioconcept.ch.

References

- Waymouth, C.; (1959) J.Nat.Cancer Inst. 22, 1003
- Waymouth, C.; (1968) Private Communication

William's E Medium

William's E Medium was developed for long term culture of liver epithelial cells of adult rats.

Cat. No.	1-48F01-I William's E Medium
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Concentration		mg/L	
Inorganic Salts			
CaCl ₂ 2H ₂ O		265	
CuSO ₄ 5H ₂ O		0.0001	
Fe(NO ₃) ₃ 9H ₂ O		0.0001	
MgSO ₄ 7H ₂ O		200	
MnCl ₄ 4H ₂ O		0.0001	
KCl		400	
NaCl		6800	
NaH ₂ PO ₄ 2H ₂ O		158	
NaHCO ₃		2200	
ZnSO ₄ 7H ₂ O		0.0002	
Other Components			
D(+)-Glucose		2000	
L-Glutathione reduced		0.05	
Methyl linolenate		0.03	
Phenol Red		10	
Pyruvic acid sodium salt		25	
Amino Acids			
Glycine		50	
L-Alanine		90	
L-Arginine		50	
L-Asparagine H ₂ O		20	
L-Aspartic acid		30	
L-Cysteine		40	
L-Cystine		20	
L-Glutamic acid		50	
L-Histidine		15	
L-Isoleucine		50	
L-Leucine		75	
Amino Acids			
L-Lysine HCl		87.6	
L-Methionine		15	
L-Phenylalanine		25	
L-Proline		30	
L-Serine		10	
L-Threonine		40	
L-Tryptophan		10	
L-Tyrosine		35	
L-Valine		50	
Vitamins			
(+)-Biotin		0.5	
Vitamin K3		0.01	
Choline chloride		1.5	
D-Pantothenic acid calcium salt		1	
Folic acid		1	
myo-Inositol		2	
Nicotinamide		1	
Pyridoxal HCl		1	
Vitamin B1 HCl		1	
Vitamin B2		0.1	
Vitamin B12		0.2	
Vitamin C		2	
Vitamin D2		0.1	
Vitamin E succinate		0.01	
Vitamin A acetate		0.1	
To be added separately			
L-Glutamine		292	

Available William's E Media

Cat. No	Modification	Size
1-48F01-I	William's E Medium w/o L-Gln	500 ml
1-48F02-I	William's E Medium w/o L-Gln, w/o Phenol Red	500 ml

Other modifications are available upon request at info@bioconcept.ch.

References

- Williams, G.M. et al.; 1974; Exp.Cell Res. 89, 139
- Williams, G.M. et al.; 1971; Exp.Cell Res. 69, 106



Special Media

MAM-PF (Production Media)

Hybridoma Express Media

Insect Cell Culture Media

BSK-H Medium for the cultivation of *Borrelia spec.*

SDM 79 for the cultivation of *Trypanosoma spec.*

Stem Cell Media

Sea anemone [*Actiniaria*]

Sea anemone is the umbrella term used for this predatory, colourful animal. They mainly live in the world's corals and are quite related to corals and jellyfish, however, unlike jellyfish, a typical sea anemone is attached to a hard surface and lacks the free swimming stage of its life cycle. They are known to make bonds with other species in their environments to improve their chance of survival, the most famous one being with the clownfish. The clownfish protects the sea anemone from predators, and in return the sea anemone offers the fish a place to call home.

Special Media

MAM-PF® for Serum-Free, Protein-Free and Animal Component Free (ACF) Cell Culture

The breakthroughs in recombinant protein production granted BioConcept Ltd. the ability to produce and supply a new type of cell culture media. BioConcept Ltd.'s MAM-PF® (Mammalian Artificial Media – Protein Free) line of media are tailor made to satisfy the needs of researchers and producers. These media for the cultivation of CHO and BHK cells are fully defined with serum-free, protein-free and ACF (Animal Component Free) versions. ACF media are produced according to EMEA/410/01 all components are certified animal component free – we hold a certificate of origin for every single component.

Products Available

Cat. No.	Description	Size	Serum free	Protein free	Animal Component free (ACF)	Formulation
Mammalian artificial Media – Animal Component free						
10-02F24-I	MAM-PF® 2 growth and production medium w/o Phenol Red	500 ml	x	x	x	Proprietary
10-02F25-I	MAM-PF® 2 growth and production medium with Phenol Red	500 ml	x	x	x	Proprietary
10-02F27-I	MAM-PF® 1 adaptation and growth medium	500 ml	x	x	x	Proprietary
10-02F33-I	MAM-PF® 2 Total Selection Medium (w/o L-Gln, L-Trp, L-His, Gly, Hypoxanthine and Thymidine)	500 ml	x	x	x	Proprietary
10-02S80-I	MAM-PF®77 production medium	500 ml	x	x	x	Proprietary
10-02P74	MAM-PF® 7e Powder Media	flexible	x	x	x	Proprietary
10-02P24	MAM-PF® 2 growth and production medium (powder) w/o Phenol Red, w/o L-Gln, w/o NaHCO ₃	1 L, 5L, 10L, 50L, 100L	x	x	x	Proprietary
5-77F01-H	Lipid-Mixture for MAM-PF® 2 Powder (1 000 x)	100 ml	x	x	x	Proprietary

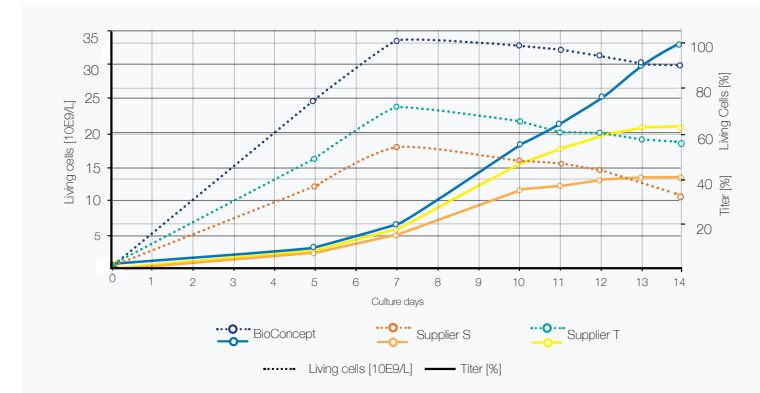
MAM-PF® Media

Mammalian Artificial Media – Protein and Animal Component Free (ACF) for CHO, BHK, and other mammalian cells

Further development of CHO-Express Media resulted in our MAM-PF® Media Series. These are chemically defined media for the cultivation of a variety of cell lines such as CHO (Chinese Hamster Ovary) Cells or BHK cells and expression of recombinant proteins in suspension cell culture. CHO and BHK cells are the most widely used mammalian cells for the expression of a variety of recombinant proteins.

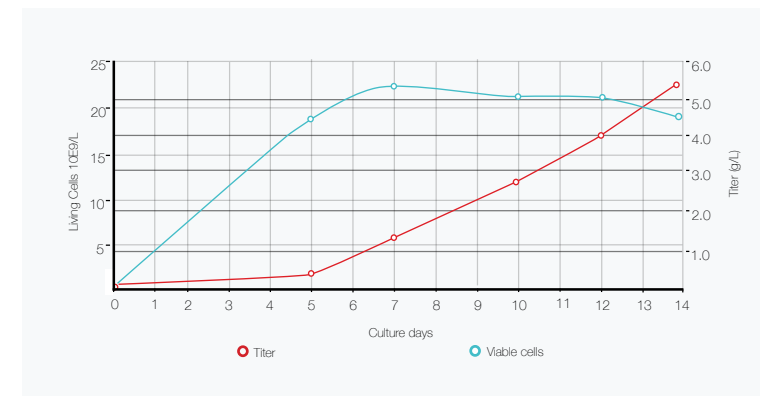
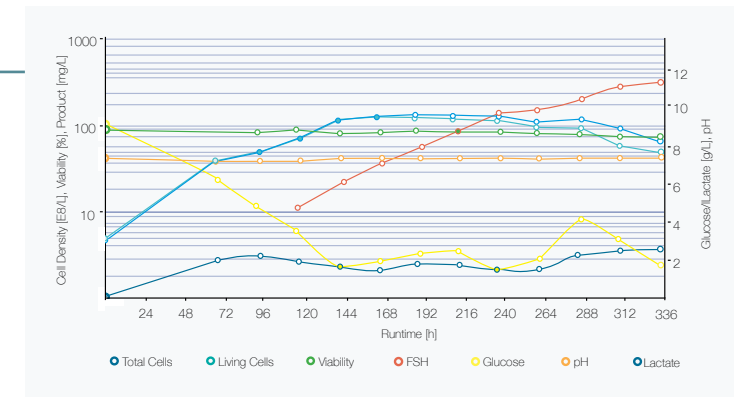
MAM-PF® Media are formulated without L-glutamine to avoid problems associated with L-glutamine degradation including ammonia accumulation. MAM-PF® Media can be obtained with a minimal amount of Phenol Red or without Phenol Red.

Serum-free culture represents an advance over serum supplemented culture as this facilitates purification and downstream processing of expressed products. Most of the commercially available serum-free media contain various undefined proteins and/or protein hydrolysates. A completely defined formulation is therefore a major advantage over both serum supplemented and serum-free systems. The absence of animal derived proteins is desirable from a regulatory standpoint and also eliminates concerns about consistency and availability. MAM-PF®1, MAM-PF®2, MAM-PF®7d and MAM-PF®7e media are chemically defined media optimized for the growth of Chinese hamster ovary (CHO) or BHK cells and expression of recombinant proteins in suspension culture. MAM-PF® media contain no proteins or peptides of animal origin and no undefined hydrolysates. L-glutamine (0.6–8.00 mM) has to be added by the end user prior to use. Various customers reported best glycosylation patterns of the recombinant expressed product when using MAM-PF® Media.



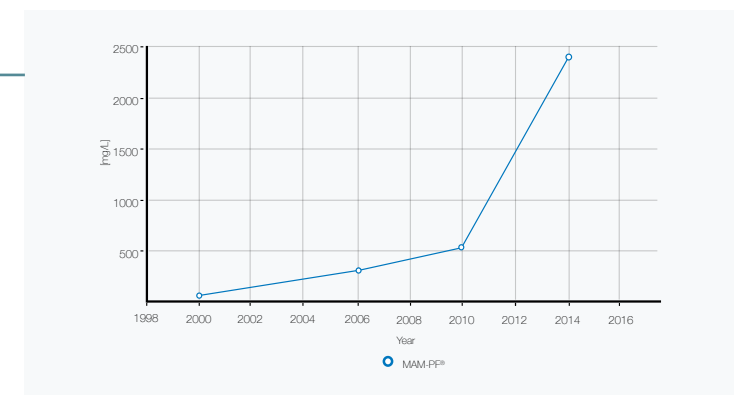
MAM-PF® and other Suppliers Performance of MAM-PF77® and two different CHO media suppliers in a 14-day fed-batch mAb production.

FSH produced with MAM-PF® and FMS3
14-day bioreactor production scheme of the high glycosylated follicle-stimulating hormone (FSH) using MAM-PF77® and the FMS3 feed mix.



Iplimumab produced with MAM-PF® and FSU
High yields of mAbs, e.g. > 5 g/L Iplimumab can be reached in a 14-day fed-batch system using MAM-PF77® plus the novel CHO feed mix FMU.

Development of MAM-PF®
Increase of Erythropoietin (EPO) yields during the system development. Within the last 4 years the product yield could be quadrupled up to 2.3 g/L using the MAM-PF77® medium and FMS3 in a fed-batch.



All graphics created by



Available MAM-PF® media, supplements and feed mixes

MAM-PF® 1 Liquid, with Phenol Red

Mammalian Artificial Medium – Animal Component Free (ACF)
Intended use: Maintenance of cells, recommended medium for adaptation.
Cat. No: 10-02F27-I, Size: 500 ml, (other sizes – up to 500 L on request)

MAM-PF® 2 Liquid, without Phenol Red

Mammalian Artificial Medium – Animal Component Free (ACF)
Intended use: Batch cultivation to achieve up to 10^7 cells/ml, production medium.
Cat. No: 10-02F24-I, Size: 500 ml, (other sizes – up to 500 L on request)

MAM-PF® 2 Powder, without Phenol Red

Mammalian Artificial Medium – Animal Component Free (ACF)
Intended use: Batch cultivation to achieve up to 10^7 cells/ml, production medium.
Cat. No: 10-02P24-K, -L, -M, -N, -W, Sizes: 1 L, 5 L, 10 L, 50 L, 100 L (other sizes on request)

MAM-PF® 2 powder is formulated without NaHCO_3 . It is supplied with a sterile supplement (Lipid Mix Cat. No: 5-77F01).

MAM-PF® 77 Liquid, without Phenol Red

Mammalian Artificial Medium – Animal Component Free (ACF)
Intended use: Batch cultivation to achieve up to 4×10^7 cells/ml, production medium.
Cat. No: 10-02S80-I, Size: 500 ml, (other sizes – up to 500 L on request)

MAM-PF® 7e Powder, without Phenol Red

Mammalian Artificial Medium – Animal Component Free (ACF)
Intended use: Batch cultivation to achieve up to 4×10^7 cells/ml, production medium.
Cat. No: 10-02P74, various quantities are available on request
The protocol for reconstitution of 10-02P74 is shown on **page 118**.

Supplementary Products

CHO-Lipid Mix (1 000×)

Intended use: CHO Lipid mixture is a 1 000× concentrate which boosts cell culture of CHO-Cells in suspension. Animal Component Free formulation (proprietary), can also be used in feeding strategies.
Cat. No: 5-74S08-H, Size: 100 ml (other sizes on request)

Lipid Mix for MAM-PF®2 powder (1 000×)

Intended use: This lipid mixture is needed as a supplement to reconstitute MAM-PF®2 powder to liquid. Animal Component Free formulation (proprietary).
Cat. No: 5-77F01-H, Size: 100 ml (other sizes on request)

CHO-Feed Mixes

MAM-PF® 6 Supplement

Intended use: Concentrate used in feeding strategies. Animal Component Free formulation (proprietary) of amino acids, vitamins and other components, no hydrolysates.
Cat. No: 5-14K06-I, Size: 500 ml (other sizes on request)

CHO-Feed Mix U1beta7

Cat. No: 5-09Z15
Sizes: 648.4 g (5-09Z15-GB), 5 kg (5-09Z15-UB), 10 kg (5-09Z15-UC)
(Customized packaging on request)

Reconstitution: use 162.1 g powder for 1 L Medium

1. Dissolve 162.1 g in 950 ml 50°C H_2O (double distilled or equivalently purified).
2. Use 19 ml 10 M NaOH to reconstitute 1 L Feed Mix. Add H_2O ad 1 L.
3. NaOH-solution may be replaced by NaOH tablets if necessary.
4. Final pH after reconstitution: 7.0–7.60. Perform sterile filtration (0.22 μm).

CHO-Feed Mix U2beta13

Cat. No: 5-09Z16
Sizes: 49.6 g (5-09Z15-AV), 100 g (5-09Z16-O), 1 kg (5-09Z16-U)
(Customized packaging on request)

Reconstitution: use 49.6 g powder for 1 L Medium

1. Dissolve 49.6 g in 950 ml H_2O (double distilled or equivalently purified). Add 14.8 g
2. NaOH tablets. Add H_2O ad 1 L. NaOH tablets may be replaced by dissolved NaOH if necessary.
3. Final pH after reconstitution: ~ 10.5–11.5. Perform sterile filtration (0.22 μm).

Usage: U2beta13 (5mL/feed*L) + U1beta7 (20mL/feed*L)

Trace Element Mixes

Intended use: Substitution of media lacking inorganic components.

Animal Component Free formulation.

Available formulations

Cat. No: 9-00K78-H/-I/-K

Cat. No: 9-00K64-H/-I/-K

Size: 100 ml/500 ml/1 L (other sizes on request)

9-00K78	9-00K64
Components	Components
CdCl ₂ ·2H ₂ O	NiSO ₄ ·6H ₂ O
ZnSO ₄ ·7H ₂ O	NH ₄ VO ₃
NaVO ₃	(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O
CoCl ₂ ·6H ₂ O	Na ₂ SiO ₃ ·5H ₂ O
CuSO ₄ ·5H ₂ O	MnSO ₄ ·H ₂ O
(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	C ₆ H ₅ FeO ₇ ·H ₂ O
BaCl ₂ ·2H ₂ O	ZnSO ₄ ·7H ₂ O
MnSO ₄ ·H ₂ O	CuSO ₄ ·5H ₂ O
SnCl ₂ ·2H ₂ O	SnCl ₂ ·2H ₂ O
NiSO ₄ ·6H ₂ O	
KI	
KBr	
CrK(SO ₄) ₂ ·12H ₂ O	
NaF	
AgNO ₃	
RbCl	
ZrOCl ₂ ·8H ₂ O	
AlCl ₃ ·6H ₂ O	
GeO ₂	
TiCl ₄	
Na ₂ SiO ₃ ·5H ₂ O	

Phenol Red (1.5 g/L)

Most MAM-PF® media are formulated without Phenol Red. If Phenol Red needs to be added: Cat. No: 5-70F01-H, Size 100 ml, Formulation: 1.5 g/L, sterile

Express Media for Hybridoma Cells

HYGM 6 and 7 Express Media

Hybridoma Growth Media (HYGM) 6 and 7 are serum free and fully defined media that can be used for the cultivation of various different hybridomas and the production of monoclonal antibodies. Both media are available with or without phenol red to prevent interference of the dye with chromogenic assays.

HYGM-6 and 7 media are ready to use.

HYGM-6 Medium is a serum free medium and contains recombinant insulin for therapeutic use. It is the only protein in that medium, no other proteins or undefined hydrolysates are present. HYGM-7 Medium is a chemically defined medium and does not contain any undefined hydrolysates or proteins.

ISF-1 Serum-free medium for Hybridoma cell culture

ISF-1 is a serum-free, chemically defined medium for hybridoma cell culture and monoclonal antibody production. It contains Glutamine and does not require further supplementation. To protect cells from shear forces during production, surfactant is included in the medium. ISF-1 is not suitable for cholesterol dependent cell lines (e.g. NSO and its variants), without further supplementation of lipoprotein.

The BSA used in ISF-1 is EDQM-certified. The addition of antibiotics should not be a substitute for proper sterile techniques. Therefore, use of antibiotics is in most cases neither necessary nor advised. However, in those instances where antibiotics are desired, ISF-1 has been shown to be compatible with the most used antibiotics (e.g. gentamycin, puromycin and amphotericin B).

Cat. No.	Description	Size	Serum free	Protein free	Animal Component free (ACF)	Formulation
Express Media for Hybridoma cells						
9-00F55-I	HYGM-6 Express, with phenol red	500 ml	×			Proprietary
9-00F57-I	HYGM-6 Express, w/o phenol red	500 ml	×			Proprietary
9-00F58-I	HYGM-7 Express, w/o phenol red	500 ml		×	×	Proprietary
9-00F67-I	HYGM-7 Express, with phenol red	500 ml		×	×	Proprietary
1-57S97-I	ISF-1 Hybridoma Growth Medium	500 ml	×		×	Proprietary

For technical information and protocols please check page 119.

Insect Cell Culture Media

General information

Nutrient requirements of insects are generally similar to those of vertebrates, but there are also some remarkable exceptions which have to be implemented when designing insect cell culture media.

Steroids

As insects have no capacity for steroidogenesis, insect cell culture media need a source for formation of cell membrane components and the steroid hormone ecdysone (Law and Wells, 1989).

Amino acids

Insect blood contains a high level of amino acids. Media for insect cell culture therefore contains high levels of amino acids (Grace 1962, Weiss et al. 1981).

Organic acids

Insect blood contains an unusually high level of free organic acids such as citrate, succinate, oxalate and malate which range from 0.1–30 mmoles per insect (Grace 1962, Vaughn 1968).

pH, buffer and pH indicator

Insect tissue fluids are more acidic and normally ranging from 6.2–6.9 (Grace 1962). Optimal range for most insect cell culture media is therefore between 6.2–6.5 compared to 7.1–7.6 for most mammalian cell culture media. SF-3 Baculo Express is optimised to keep this range under various culture conditions (e.g. open air, open capped). Insect cell culture media are buffered with sodium phosphate, no CO₂ is required to keep a constant range. No pH indicator is added to insect cell culture media. The colour of insect media is therefore yellow due to the supplementation of protein hydrolysates. In SF-3 Baculo Express these hydrolysates are added as ultrafiltrates with an 8 kDa cut off to facilitate downstream purification processes.

Osmolality

Osmotic pressure varies significantly from that in vertebrate blood, being more than twice as high (Vaughn 1968). Insect cell culture media therefore exhibit a osmolality of 340–390 mOsmol/kg compared to 290–330 mOsmol/kg for vertebrate cultures.

Glutamine/Glucose

The excess metabolism of glutamine and glucose in mammalian cell culture results in an excess production of ammonium and lactate respectively (Ljunggren and Häggström, 1992), and accumulation of these metabolic byproducts is often inhibitory. Detoxification of these metabolites in insect cells follows a different pathway than in mammalian cells. Therefore higher levels of Glutamine and Glucose can be used in insect cell culture media to support high cell densities growth (reviewed in: Schläger, 1996).

Continued on page 54

Available Insect Cell Culture Media

Cat. No.	Description	Size	Serum free	Protein free	Formulation
9-00F38-I	SF-4 Baculo Express ICM «ready to use»	500 ml	×	Contains yeast extract as only undefined component	Proprietary
9-00F38-K	SF-4 Baculo Express ICM «ready to use»	1 L	×	Contains yeast extract as only undefined component	Proprietary
9-07S38-I	SF-4 Baculo Express (1.1 × conc.) w/o yeast extract, w/o L-Valine	500 ml	×	×	Proprietary
9-10S38-I	SF-4 Baculo Express (1.1 × conc.) w/o yeast extract, w/o L-Methionine	500 ml	×	×	Proprietary
9-05S38-I	SF-4 Baculo Express (1.1 × conc.) w/o yeast extract, w/o L-Tyrosine	500 ml	×	×	Proprietary
9-02S38-I	SF-4 Baculo Express (1.1 × conc.) w/o yeast extract, w/o amino acids	500 ml	×	×	Proprietary
1-12F20-I	TC-100 Insect Cell Culture Medium	500 ml	Heat Inactivated Serum needs to be added		
1-12F07-I	Grace's Insect Cell Culture Medium	500 ml	Heat Inactivated Serum needs to be added		See page 56
1-43F00-I	Schneider's <i>Drosophila</i> Medium w/o L-Gln	500 ml		×	See page 59
1-43F02-I	Schneider's <i>Drosophila</i> Medium with L-Gln	500 ml		×	See page 59
1-34F00-I	Mitsuhashi and Maramorosh	500 ml	Contains Lactalbumin-hydrolysate and yeast extract		See page 58

Other modifications of SF-4 Media are available at info@bioconcept.ch.

SF-Baculo Express Media

Extensive further development and further investigation on the nutritional needs of insect cells, based on the excellent performance of SF-1, resulted in our new improved “Ready to Use” insect medium. Already successfully used in different academic and industrial laboratories, SF-4 show following improvements:

1. Cell density: Densities up to 2×10^7 cells/ml could be achieved using SF-4 in bioreactors and spinner flasks
2. Versatility: Not only suitable for SF9 and SF21 but also High Five™ and *Drosophila* cells
3. Adaptation: Only few passages are needed, if you switch from your current serum supplemented medium (e.g. TC-100 or Grace's). Direct switch from your current serum free (but not protein free) medium is possible for some of the commercially available media.
4. Protein yield: Results indicate an increased protein yield (1.5–2.7 times) in recombinant protein production compared to previously used media.

SF-4 Baculo Express ICM (Insect Culture Medium)

9-00F38, Ready to use, no supplementation required. Formulation proprietary.

SF-4 Baculo express medium is a proprietary formulation which has successfully been used to grow various *Spodoptera frugiperda* (SF9, SF21), BTI-TN-5B1-4 (High Five™) and *Drosophila melanogaster* (D.Mel-2) cells.

Amino acid depleted SF-4 medium (Cat. No: 9-02S38-I, 9-05S38-I, 9-10S38-I) is an efficient reagent for isotope labeling in NMR studies (see References **page 55**).

Other modifications are available upon request at info@bioconcept.ch.

For technical information and protocols please check **page 120**.

References

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Grace's Insect TC Medium

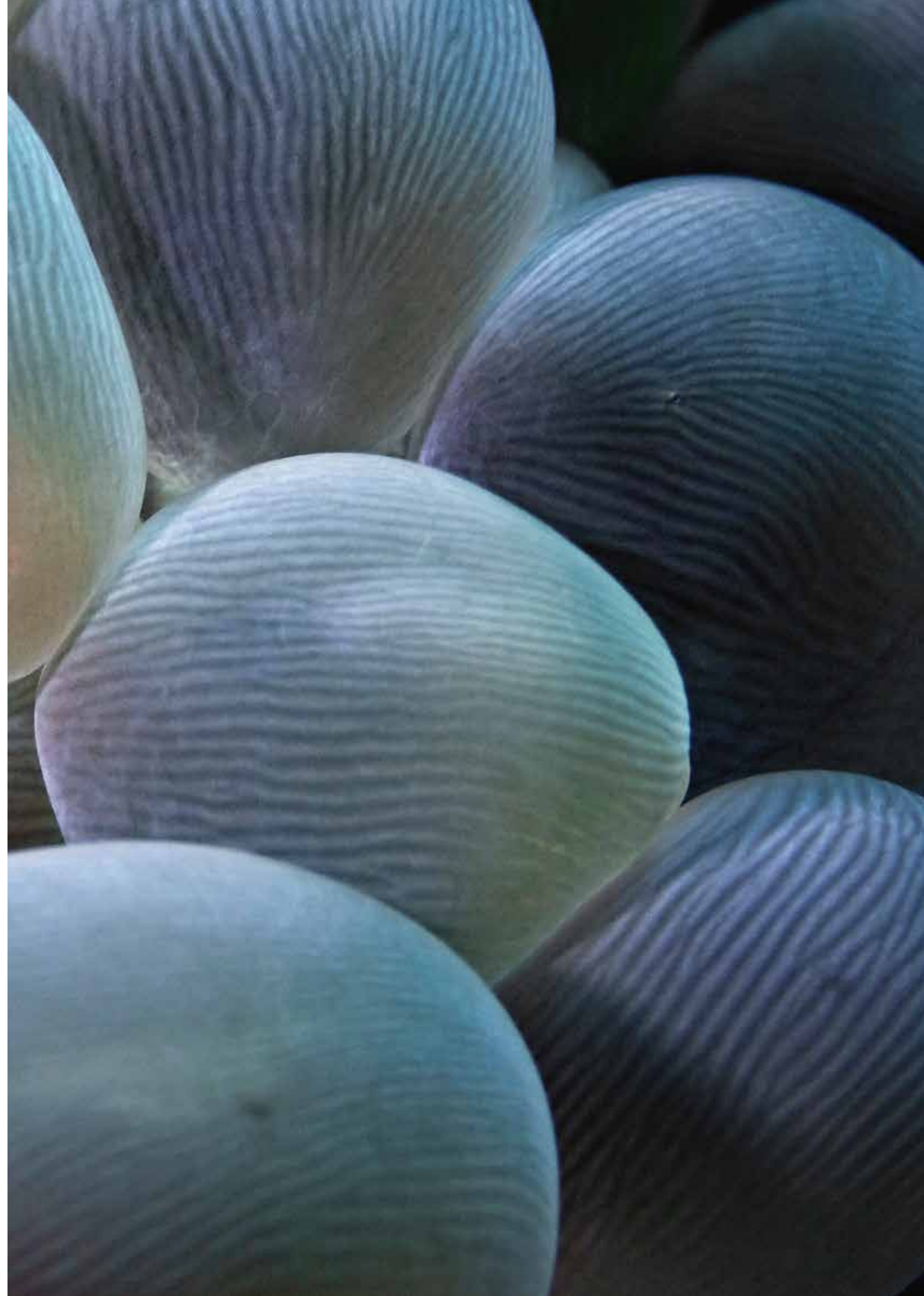
Grace's Medium has always been a popular medium for insect cell culture. The medium was formulated to resemble the chemical composition of the hemolymph from *Bombyx mori*. Prior to use, the medium is typically supplemented with FCS, yeast extract, lactalbumin hydrolysate and BSA in varying combinations and amounts. The medium is generally used to culture *dipteran* and *lepidopteran* cell lines.

Grace's Insect Medium 1-12F07-I

Concentration			
Inorganic Salts		mg/L	Amino Acids
CaCl ₂ anhydrous	750	L-Isoleucine	50
KCl	4100	L-Leucine	75
MgCl ₄ 6H ₂ O	2280	L-Lysine HCl	625
MgSO ₄ 7H ₂ O	2780	L-Methionine	50
NaHCO ₃	350	L-Phenylalanine	150
NaH ₂ PO ₄ H ₂ O	1013	L-Proline	350
Other Components		DL-Serine	1100
α-Ketoglutarate	370	L-Threonine	175
Fructose	400	L-Tryptophan	100
Fumarate	55	L-Tyrosine	50
D-Glucose	700	L-Valine	100
Maleinate	670	Vitamins	
D-Saccharose	26680	Biotin	0.01
D-Succinate	60	D-Ca Pantothenate	0.02
Amino Acids		Choline Chloride	0.2
β-Alanine	200	Folic Acid	0.02
L-Alanine	225	i-Inositol	0.02
L-Arginine HCl	700	Niacin	0.02
L-Asparagine	350	Para Aminobenzoic Acid	0.02
L-Aspartic Acid	350	Pyridoxin HCl	0.02
L-Cystine	22	Riboflavin	0.02
L-Glutamic Acid	600	Thiamine HCl	0.02
Glycine	650	To be added separately	
L-Histidine HCl H ₂ O	3378	L-Glutamine	600

References

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Mitsuhashi/Maramorosh Insect Medium

Mitsuhashi and Maramorosh basal medium is suited for mosquito cell culture. The complete medium has to be supplemented with FCS prior to use.

Mitsuhashi/Maramorosh Insect Medium 1-34F00-I

Concentration		Other Components	
Inorganic Salts	mg/L		mg/L
CaCl ₂ 2H ₂ O	200	D-Glucose	4000
KCl	200	Lactalbumin Hydrolysate	6500
MgCl ₂ 6H ₂ O	100	Yeastolate	5000
NaCl	7000		
NaHCO ₃	120		
NaH ₂ PO ₄ H ₂ O	200		

References

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Schneider's *Drosophila* Medium

This Medium was originally developed for the culture of *Drosophila* cells. However, it can also be used for the culture of other dipteran cell lines.

Schneider's *Drosophila* Medium 1-43F00-I

Concentration		Amino Acids	
Inorganic Salts	mg/L		mg/L
CaCl ₂ 2H ₂ O	795	L-Cysteine	60
KCl	1600	L-Cystine	100
KH ₂ PO ₄	450	L-Glutamic Acid	800
MgSO ₄ 7H ₂ O	3700	Glycine	250
NaCl	2100	L-Histidine	400
NaHCO ₃	400	L-Isoleucine	150
Na ₂ HPO ₄	700	L-Leucine	150
Other Components		L-Lysine HCl	1650
α-Ketoglutarate	200	L-Methionine	800
Fumarate	100	L-Phenylalanine	150
D-Glucose	2000	L-Proline	1700
Maleinate	100	L-Serine	250
D-Succinate	100	L-Threonine	350
Trehalose	2000	L-Tryptophan	100
Yeastolate	2000	L-Tyrosine	500
Amino Acids		L-Valine	300
α-Alanine	500	To be added separately	
L-Arginine HCl	400	L-Glutamine	1800
L-Aspartic Acid	400		

Cat. No: 1-43F02-I contains L-Glutamine.

References

- Schneider, Imogene (1964) Exp. Zool. 156: 1:91
- Schneider, I. and Blumental (1978) *Drosophila* Cell and Tissue Culture. In: Biology and Genetics of *Drosophila* vol. 2A, M. Ashburner and T.R.F. Wright eds., Academic Press, N.Y., pp. 266–315

BSK-H Medium for the Cultivation of *Borrelia* Spec.

BSK-H medium is a high quality nutrient liquid for the reliable cultivation of *Borrelia* spec. BSK-H medium is a quite complex formulation with an extraordinary high content of proteins and peptides. It is especially rich in nucleosides, glucose as energy source and contains high concentrations of vitamins. The medium contains N-Acetyl-D-Glucosamine which is an essential element of the bacterial peptidoglycan.

Only selected reagents of highest quality are used for the production of BSK-H medium. A special galenic as well as a gentle and controlled process guarantee a product of highest quality with reliable lot to lot constancy, stability, purity and reliable cell growth. A sufficient high amount of HEPES guarantees a stable buffer capacity for a long time. The concentration of CO₂ as well as the pH value have to be controlled carefully, because in some cases, due to metabolisation of glucose in long-term cultures, lactic acid accumulation occurs, which may result in a reduction of the pH-value of more than 1 pH unit. At room temperature the pH value is 7.6±0.2 at an osmolality of 420±20 mOsm/kg H₂O. The ready-to-use medium has to be supplemented with 3–8 % rabbit serum prior to inoculation. Storage temperature and stability are according to lot-specific label.

BSK-H medium can be widely used for the cultivation of *spirochetes*, especially for *B. burgdorferi* and *B. hermsii*. Only small amounts of organisms are sufficient for the inoculation. However, the values found in the literature, as well as personal communications are varying too much, so that no general recommendation for the inoculum number can be given. The generation time lies between 11 and 18 hours, so that in 7 to 9 days 0.5–4.0×10⁸ cells/ml can be obtained. The optimal incubation temperature lies between 30 °C and 37 °C.

Formulation of BSK-H Medium

BSK-H Medium <i>Borrelia</i>		1-10S03-I	
Concentration			
Inorganic Salts	mg/L	Amino Acids	mg/L
CaCl ₂ 2H ₂ O	264.92	L-Phenylalanine	25
KCl	400	L-Proline	40
MgSO ₄ 7H ₂ O	200	L-Serine	25
NaCl	6800	L-Threonine	30
NaHCO ₃	2200	L-Tryptophan	10
NaH ₂ PO ₄ H ₂ O	140	L-Tyrosine	40
Other Components		L-Valine	25
N-Acetyl-D-Glucosamine	400	Vitamins	
Coccarboxylase 4H ₂ O	1	Asorbic Acid	50
Coenzyme A 3H ₂ O	2.5	Biotin	0.01
Albumin	50000	D-Ca Pantothenate	0.01
Neopeptone	5000	Cholesterol	0.20
Yestolate	2000	Choline Chloride	0.50
D-Glucose	6000	Folic Acid	0.01
Glutathione Reduced	10	i-Inositol	0.05
Sodium Acetate anhydrous	50	Niacinamide	0.025
Sodium Glucuronate	4.2	Niacin	0.025
Citric Acid H ₂ O	500	Paraaminobenzoic Acid	0.05
Pyruvic Acid	800	Pyridoxal HCl	0.025
HEPES	6000	Pyridoxin HCl	0.025
Tween 80	5	Riboflavin	0.01
Phenol Red	20	Thiamine	0.01
Amino Acids		Nucleosides / Nucleotides	
L-Alanine	25	Deoxyadenosine	10
L-Arginine HCl	70	Deoxycytidine HCl	11.6
L-Aspartic Acid	30	Deoxyguanosine	10
L-Cysteine HCl H ₂ O	260	Thymidine	10
L-Cystine	20	5-Methyldeoxycytidine	0.1
L-Glutamic Acid	75	FlavinAdenine Dinucleotide	1
L-Glycine	50	NAD	7
L-Histidine HCl H ₂ O	20	β-NADP 2Na	1
Trans-4-Hydroxy-L-Proline	10	Uridine-5-Triphosphate 3Na	1
L-Isoleucine	20	To be added separately	
L-Leucine	60	Rabbit Serum	3–8 %
L-Lysine HCl	70	To be added optionally	
L-Methionine	15	L-Glutamine	100–300*

*BSK-H Medium with 300 mg/L L-Glutamine, Cat. No: 1-10S02-I, Size: 500 ml

Note:

Prior to inoculation with bacteria, medium and serum should be heated to 30–32 °C.

SDM 79 For the Cultivation of *Trypanosoma Spec.*

Brun and Jenni (1979) developed a semi-defined medium for *Trypanosoma brucei*, which has successfully been used to culture various species of *Trypanosoma*. This medium is available in both liquid and powder.

Cat. No.	Description	Size
9-03V02-K, -L, -M, -N	SM Medium (Powder)*	1 L, 5 L, 10 L, 50 L
9-04S22-I	SDM79 (liquid)	500 ml
9-04V01-K, -L, -M, -N	SDM79 (Powder)	1 L, 5 L, 10 L, 50 L

*SM Medium for *T. Brucei procyclic forms* (formulation according to Cunningham)

Formulation of SDM79

Concentration			
Inorganic Salts		mg/L	Amino Acids
CaCl ₂ anhyd.		189.21	L-Phenylalanine
Fe(NO ₃) ₃ 9H ₂ O		0.15	L-Proline
KCl		378.43	L-Threonine
MgSO ₄ anhyd.		92.39	L-Serine
NaCl		6433.26	L-Tryptophan
NaHCO ₃		2000	L-Tyrosine 2 Na-Salt
NaH ₂ PO ₄ H ₂ O		132.45	L-Valine
Other Components			Vitamins
D (+) Glucosamine HCl		50	Asorbic Acid
Taurine		180	Thiamine HCl
D-Ribose		0.105	Biotin
Hypoxanthine		0.063	D-Ca Pantothenate
Desoxy-D-Ribose		0.105	Cholesterol
Tween 80		4.19	Choline Chloride
D-Glucose		1946.07	Folic Acid
Glutathione Reduced		0.01	i-Inositol
Sodium Acetate anhyd.		10.48	Niacinamide
Sodium Pyruvate		100	Niacin
HEPES		8000	Paraaminobenzoic Acid
MOPS		5000	Pyridoxal HCl
Phenol Red		11.56	Pyridoxin HCl
Amino Acids			Riboflavin
L-Alanine		210.58	DL-alpha-Tocopherol succinate
L-Arginine HCl		229.20	Ergocalciferol
L-Asparagine H ₂ O		9	Vitamin A Acetate
L-Aspartic Acid		14.27	Menadion
L-Cysteine HCl H ₂ O		0.023	Nucleosides and Precursors
L-Cystine 2HCl		28.63	Adenosine
L-Glutamine		536.01	Adenine Sulfate
L-Glutamic Acid		22.82	Guanosine
L-Glycine		14.98	Guanine HCl
L-Histidine HCl H ₂ O		35.63	Xanthine
Trans-4-Hydroxy-L-Proline		2.10	Uracil
L-Isoleucine		46.89	Thymin
L-Leucine		51.08	AMP H ₂ O
L-Lysine HCl		68.77	ATP 2Na H ₂ O
L-Methionine		84.18	

References

Brun R. and Schonenberger M. (1979) Cultivation and in vitro cloning of procyclic culture forms of *Trypanosoma brucei* in a semi-defined medium. Acta Trop,36:289–92.

Stem Cell Media and Supplements

BIT 9500 Serum Substitute

Developed for use in serum-free culture conditions with defined composition. Contains bovine serum albumin (BSA), insulin and transferrin in Iscove's IMDM.

Product Properties:

- Store at -20°C .
- pH is set at 7.1–7.5 and osmolality at 300 ± 20 mOsm/kg.
- Thaw at room temperature ($15-20^{\circ}\text{C}$) or overnight at $2-8^{\circ}\text{C}$. Swirl the bottle to mix its content. Store at $2-8^{\circ}\text{C}$ for up to 1 month. Alternatively, aliquot and store at -20°C . After thawing the aliquots, do not refreeze. Use BIT 9500 at a final concentration of 20 %.
- Contains bovine serum albumin, recombinant human insulin, human transferrin (iron-saturated), Iscove's IMDM.
- This product should be considered potentially infectious and treated in accordance with universal handling precautions.
- Not intended for any human or animal diagnostic or therapeutic use.

Human Mesenchymal Stem Cell (hMSC) proliferation medium

Media for culturing hMSC delivered as a basal medium with supplements. The formulation is optimized for initial seeding of 6000 cells/cm² up to a confluence of approximately 90 %. Feeder-layer, matrix substrates or other substances are not necessary. Due to the possibility of reduced proliferative activity, we recommend the use of antibiotic supplement for freshly isolated cells only.

Product Properties:

- Place the bottle of basal medium in the dark at $4-8^{\circ}\text{C}$ immediately after delivery. Store the supplements at -20°C .
- The supplemented hMSC proliferation growth medium can be stored in the dark at $4-8^{\circ}\text{C}$ for up to 1 month. Do not heat the medium over 37°C or use uncontrollable heat sources (e.g. microwave appliances). If only part of the medium is to be used, remove the required amount from the bottle and heat it.
- Do not freeze the medium. This can lead to high salt concentrations by freezing out pure water, which will cause irreversible damage.
- pH is set at 7.2–7.6 and osmolality at 285 ± 10 mOsm/kg.
- Not intended for any human or animal diagnostic or therapeutic use.

Cat. No	Modification	Size
11-01F03-I	hMSC proliferation medium, basal	500 ml
11-01F04-KIT	hMSC proliferation medium FCS kit	Kit
11-01F05-KIT	hMSC proliferation medium FCS supplement kit	Kit

Human Mesenchymal Stem Cell (hMSC) chondrogenesis induction medium

The hMSC chondrogenesis induction medium induces chondrogenic differentiation of hMSCs. The medium is delivered as a basal medium with supplements. The formulation is optimized for initial seeding of 6000 cells/cm² up to a confluence of approximately 90 %. Feeder-layer, matrix substrates or other substances are not necessary. Due to the possibility of reduced proliferative activity, we recommend the use of antibiotic supplement for freshly isolated cells only.

Product Properties:

- Place the bottle of basal medium in the dark at $4-8^{\circ}\text{C}$ immediately after delivery. Store the separately delivered supplements at -20°C or 4°C , according to the instructions on the vials.
- After adding 10 ml of HEPES to the basal medium, it can be stored in the dark at $4-8^{\circ}\text{C}$ for up to 4 weeks. After adding optional 3,5 ml of antibiotics to the basal medium, it can be stored in the dark at $4-8^{\circ}\text{C}$ for up to 1 week. Therefore, each aliquot contains enough volume to prepare 50 ml hMSC chondrogenesis induction media only. To prepare 50 ml chondrogenesis induction media add 500 μl ITS, 500 μl Dexamethasone, 500 μl Sodium pyruvate, 500 μl Ascorbic-acid-2-phosphate and 500 μl Proline to 50 ml hMSC chondrogenesis induction basal medium (already supplemented with HEPES). 500 μl TGF- β 3 must be added always fresh. Do not use the supplemented media for longer than one week. Do not heat the medium over 37°C or use uncontrollable sources of heat (e.g. microwave appliances). If only part of the medium is to be used, remove the required amount from the bottle and heat it.
- Do not freeze the medium. This can lead to high salt concentrations by freezing out pure water, which will cause irreversible damage.
- pH is set at 7.2–7.6 and osmolality at 285 ± 10 mOsm/kg.
- Not intended for any human or animal diagnostic or therapeutic use.

Cat. No	Modification	Size
11-01F08-I	hMSC chondrogenesis induction medium basal, serum free	500 ml
11-01F09-KIT	hMSC chondrogenesis induction kit, serum free	Kit

For technical information and protocols please check **page 122**.

Human Mesenchymal Stem Cell (hMSC) osteogenesis induction medium

The hMSC osteogenesis induction medium induces osteogenic differentiation of hMSCs. The medium is delivered as a basal medium with supplements. The formulation is optimized for initial seeding of 6000 cells/cm² up to a confluence of approximately 90%. Feeder-layer, matrix substrates or other substances are not necessary. Due to the possibility of reduced proliferative activity, we recommend the use of antibiotic supplement for freshly isolated cells only.

Product Properties:

- Place the bottle of basal medium in the dark at 4–8 °C immediately after delivery. Store the separately delivered supplements at –20 °C.
- After adding 50 ml of FCS, 10 ml of HEPES and 5 ml of L-Glutamine and optional 3,5 ml of antibiotics to the basal medium, it can be stored in the dark at 4–8 °C for up to 4 weeks. After adding the osteogenesis induction factors Dexamethasone, Ascorbic-acid-2-phosphate and β-glycerol-phosphate to the media, the media can be stored in the dark at 4–8 °C for up to 1 week. Therefore, each aliquot contains enough volume to prepare 50 ml hMSC osteogenesis induction media only. To prepare 50 ml osteogenesis induction media add 500 μl Dexamethasone, 500 μl Ascorbic-acid-2-phosphate and 500 μl β-glycerol-phosphate to 50 ml hMSC osteogenesis induction basal medium (already supplemented with FCS, HEPES and L-Glutamine). Do not use the supplemented media for longer than one week. Do not heat the medium over 37 °C or use uncontrollable sources of heat (e.g. microwave appliances). If only part of the medium is to be used, remove the required amount from the bottle and heat it.
- Do not freeze the medium. This can lead to high salt concentrations by freezing out pure water, which will cause irreversible damage.
- pH is set at 7.2–7.6 and osmolality at 285 ± 10 mOsm/kg.
- Not intended for any human or animal diagnostic or therapeutic use.

Cat. No	Modification	Size
11-01F10-I	hMSC osteogenesis induction medium, basal	500 ml
11-01F11-KIT	hMSC osteogenesis induction medium FCS kit	Kit
11-01F12-KIT	hMSC osteogenesis induction medium FCS supplement kit	Kit

For technical information and protocols please check **page 122**.

Human Mesenchymal Stem Cell (hMSC) adipogenesis induction medium

The hMSC adipogenesis induction medium induces adipogenesis differentiation of hMSCs. The medium is delivered as a basal medium with supplements. The formulation is optimized for initial seeding of 6000 cells/cm² up to a confluence of approximately 90%. Feeder-layer, matrix substrates or other substances are not necessary. Due to the possibility of reduced proliferative activity, we recommend the use of antibiotic supplement for freshly isolated cells only.

Product Properties:

- Place the bottle of basal medium in the dark at 4–8 °C immediately after delivery. Store the separately delivered supplements at –20 °C.
- After adding 50 ml of FCS, 10 ml of HEPES and 5 ml of L-Glutamine and optional 3,5 ml of antibiotics to the basal medium, it can be stored in the dark at 4–8 °C for up to 4 weeks. After adding the adipogenesis induction factors Dexamethasone, Indomethacine, 3-isobutyl-1-methyl-xanthine and Insulin to the media, the media can be stored in the dark at 4–8 °C for up to 1 week. Therefore, each aliquot contains enough volume to prepare 50 ml hMSC adipogenesis induction media only. To prepare 50 ml adipogenesis induction media add 500 μl Dexamethasone, 500 μl Indomethacine, 500 μl 3-isobutyl-1-methyl-xanthine and 500 μl Insulin to 50 ml hMSC adipogenesis induction basal medium (already supplemented with FCS, HEPES and L-Glutamine). Do not use the supplemented media for longer than one week. Do not heat the medium over 37 °C or use uncontrollable sources of heat (e.g. microwave appliances). If only part of the medium is to be used, remove the required amount from the bottle and heat it.
- Do not freeze the medium. This can lead to high salt concentrations by freezing out pure water, which will cause irreversible damage.
- pH is set at 7.2–7.6 and osmolality at 285 ± 10 mOsm/kg.
- Not intended for any human or animal diagnostic or therapeutic use.

Cat. No	Modification	Size
11-01F06-I	hMSC adipogenesis induction medium, basal	500 ml
11-01F07-KIT	hMSC adipogenesis induction medium FCS supplement kit	Kit

For technical information and protocols please check **page 123**.

Cancer Stem Cell Medium

Media for culturing human cancer stem cells delivered as a basal medium with supplements. The formulation is optimized for initial seeding of 6000 cells/cm² up to a confluence of approximately 90%. Feeder-layer, matrix substrates or other substances are not necessary. Due to the possibility of reduced proliferative activity, we recommend the use of antibiotic supplement for freshly isolated cells only.

Product Properties:

- Place the bottle of basal medium in the dark at 4–8 °C immediately after delivery. Store the supplements at –20 °C.
- The supplemented cancer stem cell medium can be stored in the dark at 4–8 °C for up to 1 month. Do not heat the medium over 37 °C or use uncontrollable heat sources (e.g. microwave appliances). If only part of the medium is to be used, remove the required amount from the bottle and heat it.
- Do not freeze the medium. This can lead to high salt concentrations by freezing out pure water, which will cause irreversible damage.
- pH is set at 7.2–7.6 and osmolality at 285 ± 10 mOsm/kg.
- Not intended for any human or animal diagnostic or therapeutic use.

Cat. No	Modification	Size
11-01F01-I	Cancer stem cell medium, basal, serum free	500 ml
11-01F02-KIT	Cancer stem cell medium kit, serum free	Kit



Salt Solutions / Buffers / Reagents & Supplements

Salt Solutions / Buffers /
Cell Culture Water

Media Supplements

Trypsin Solutions

Antibiotic / Antimycotic Solutions

Mandarinfish [*Synchiropus Splendidus*]

The mandarinfish is a small but vibrant fish that can be found in the Pacific, usually in warmer waters around the south coast of Australia.

Due to its bright colours the fish is very popular in the aquarium trade, despite the fact they are notoriously difficult to keep as they are very fussy eaters. Interestingly, the fish does not have scales, but is instead covered by a layer of tiny spines and a thick covering of slime.

The slime is not only toxic and gives the fish an awful taste, but it also smells disgusting, working as an effective deterrent to predators.

Salt Solutions / Buffers / Cell Culture Water

20 × SSC und 10 × TBE

Cat. No.	3-07F00-I	3-07F01-I
Unit Size	500 ml	500 ml
Presentation (liquid)	20 × SSC	10 × TBE
Formula	according to Maniatis	according to Maniatis
Concentration		
Components	g/L	g/L
Tri-Sodiumcitrate 2H ₂ O	88.23	–
NaCl	175.32	–
Tris (hydroxymethyl) aminomethane	–	121.14
Boric acid	–	55.647
EDTA 2Na 2H ₂ O	–	37.224

Earle's Balanced Salt Solution EBSS

Cat. No.	3-01F29-I	3-01F33-I	3-01F00-I	3-01F31-I
Unit Size	500 ml	500 ml	500 ml	500 ml
Presentation	1 × liquid	1 × liquid	1 × liquid	1 × liquid
Formula	w/o Ca ⁺⁺ /Mg ⁺⁺	w/o Ca ⁺⁺ /Mg ⁺⁺ w/o Phenol Red	with Ca ⁺⁺ /Mg ⁺⁺	with Ca ⁺⁺ /Mg ⁺⁺ w/o Phenol Red
Concentration				
Inorganic Salts	g/L	g/L	g/L	g/L
CaCl ₂ 2H ₂ O	–	–	0.264	0.264
KCl	0.40	0.40	0.40	0.40
MgSO ₄ 7H ₂ O	–	–	0.20	0.20
NaCl	6.80	6.80	6.80	6.80
NaHCO ₃	2.20	2.20	2.20	2.20
NaH ₂ PO ₄ H ₂ O	0.14	0.14	0.14	0.14
Other Components				
D-Glucose	1.00	1.00	1.00	1.00
Phenol Red	0.01	–	0.01	–

Cat. No.	3-01K30-I	3-01K34-I
Unit Size	500 ml	500 ml
Presentation	10 × liquid	10 × liquid
Formula	w/o Ca ⁺⁺ /Mg ⁺⁺ w/o NaHCO ₃ w/o NaHCO ₃	w/o Ca ⁺⁺ /Mg ⁺⁺ w/o Phenol Red
Concentration		
Inorganic Salts	g/L	g/L
KCl	4.00	4.00
NaCl	68.00	68.00
NaHCO ₃	–	–
NaH ₂ PO ₄ · H ₂ O	1.4	1.4
Other Components		
D-Glucose	10.00	10.00
Phenol Red	0.1	–

Hank's Balanced Salt Solution (HBSS)

Cat. No.	3-02F00-I	3-02F31-I	3-02F29-I	3-02F33-I
Unit Size	500 ml	500 ml	500 ml	500 ml
Presentation	1 × liquid	1 × liquid	1 × liquid	1 × liquid
Formula	Standard	w/o Phenol Red	w/o Ca ⁺⁺ /Mg ⁺⁺	w/o Ca ⁺⁺ /Mg ⁺⁺ w/o Phenol Red
Concentration				
Inorganic Salts	g/L	g/L	g/L	g/L
CaCl ₂ · 2H ₂ O	0.185	0.185	–	–
KCl	0.40	0.40	0.40	0.40
KH ₂ PO ₄	0.06	0.06	0.06	0.06
MgSO ₄	0.20	0.20	–	–
NaCl	8.00	8.00	8.00	8.00
NaHCO ₃	0.35	0.35	0.35	0.35
Na ₂ HPO ₄	0.048	0.048	0.048	0.048
Other Components				
D-Glucose	1.00	1.00	1.00	1.00
Phenol Red	0.01	–	0.01	–

Cat. No.	3-02K02-I	3-02K32-I	3-02K30-I	3-02K34-I
Unit Size	500 ml	500 ml	500 ml	500 ml
Presentation	10 × liquid	10 × liquid	10 × liquid	10 × liquid
Formula	Standard	w/o Phenol Red	w/o Ca ⁺⁺ /Mg ⁺⁺	w/o Ca ⁺⁺ /Mg ⁺⁺ w/o Phenol Red
Concentration				
Inorganic Salts	g/L	g/L	g/L	g/L
CaCl ₂ · 2H ₂ O	1.85	1.85	–	–
KCl	4.00	4.00	4.00	4.00
KH ₂ PO ₄	0.60	0.60	0.60	0.60
MgSO ₄ · 7H ₂ O	2.00	2.00	–	–
NaCl	80.00	80.00	80.00	80.00
Na ₂ HPO ₄	0.48	0.48	0.48	0.48
Other Components				
D-Glucose	10.00	10.00	10.00	10.00
Phenol Red	0.10	–	0.10	–
To be added separately				
NaHCO ₃	3.50	3.50	3.50	3.50

Cat. No.	3-02P02 -K, -L, -M, -N	3-02P32 -K, -L, -M, -N	3-02P30 -K, -L, -M, -N
Unit Size	1 L, 5 L, 10 L, 50 L	1 L, 5 L, 10 L, 50 L	1 L, 5 L, 10 L, 50 L
Presentation	powder	powder	powder
Formula	Standard	w/o Phenol Red	w/o Ca/Mg
Concentration			
Inorganic Salts	g/L	g/L	g/L
CaCl ₂ anhydrous	0.14	0.14	–
KCl	0.40	0.40	0.40
KH ₂ PO ₄	0.06	0.06	0.06
MgSO ₄ anhydrous	0.098	0.098	–
NaCl	8.00	8.00	8.00
Na ₂ HPO ₄	0.048	0.048	0.048
Other Components			
D-Glucose	1.00	1.00	1.00
Phenol Red	0.01	–	0.01
To be added separately			
NaHCO ₃	0.35	0.35	0.35

Phosphate Buffered Saline (pH 7.4)

Cat. No.	3-05F39-I
Unit Size	500 ml
Presentation	1 × liquid
Concentration	
Inorganic Salts	g/L
KH ₂ PO ₄	0.144
Na ₂ HPO ₄ 7H ₂ O	0.795
NaCl	9.00

Physiological (0.9%) Sodium Chloride Solution

Cat. No.	3-06S00-I
Unit Size	500 ml
Presentation	1 × liquid
Concentration	
Inorganic Salts	g/L
NaCl	90.00

Dulbecco's PBS

Cat. No.	3-05F00-I	3-05F29-I/-K	3-05K00-I	3-05K29-I
Unit Size	500 ml	500 ml, 1 L	500 ml	500 ml
Presentation	1 × liquid	1 × liquid	10 × liquid	10 × liquid
Formula	Standard	w/o Ca ⁺⁺ /Mg ⁺⁺	Standard	w/o Ca ⁺⁺ /Mg ⁺⁺
Concentration				
Inorganic Salts	g/L	g/L	g/L	g/L
CaCl ₂ 2H ₂ O	0.132	–	1.32	–
KCl	0.20	0.20	2.00	2.00
KH ₂ PO ₄	0.20	0.20	2.00	2.00
MgCl ₂ 6H ₂ O	0.10	–	1.00	–
NaCl	8.00	8.00	80.00	80.00
Na ₂ HPO ₄	1.15	1.15	11.50	11.50

Cat. No.	8-05F00-I	8-05F29-I	3-05P00 -K, -L, -M, -N	3-05P29 -K, -L, -M, -N
Unit Size	500 ml	500 ml	1L, 5L, 10L, 50L	1L, 5L, 10L, 50L
Presentation	1 × liquid	1 × liquid	powder	powder
Formula	Standard Endotoxin free	w/o Ca ⁺⁺ /Mg ⁺⁺ Endotoxin free	Standard	w/o Ca ⁺⁺ /Mg ⁺⁺
Concentration				
Inorganic Salts	g/L	g/L	g/L	g/L
CaCl ₂ 2H ₂ O	0.132	–	–	–
KCl	0.20	0.20	0.20	0.20
KH ₂ PO ₄	0.20	0.20	0.20	0.20
MgCl ₂ 6H ₂ O	0.10	–	0.10	–
NaCl	8.00	8.00	8.00	8.00
Na ₂ HPO ₄	1.15	1.15	–	–
To be added seperately				
CaCl ₂ anhydrous	–	–	0.1	–

EDTA PBS (0.02%)

Cat. No.	5-32F00-H/-I
Unit Size	100 ml/500 ml
Presentation	0.02% solution
Formula	w/o Ca ⁺⁺ /Mg ⁺⁺

Sodium Bicarbonate Solution

Cat. No.	5-30F00-H/-I
Unit Size	100 ml/500 ml
Presentation	7.5% solution
Formula	75 g/L

Water for Cell Culture (Endotoxin Free)

Cat. No.	8-73F00-I/-K/-M
Unit Size	500 ml/1 L/10 L
Presentation	liquid
Formula	Endotoxin free

HEPES Buffer

Cat. No.	5-31F00-H
Unit Size	100 ml
Presentation	1 M solution
Formula	238.3 g/L

HEPES Powder (Free Acid)

Cat. No.	5-31P00-O/-R
Unit Size	100 g/500 g
Presentation	powder
Formula	free acid

Red Blood Cell (RBC) Lysis Buffer

Cat. No.	3-13F00-H
Unit Size	100 ml
Presentation	liquid
Formula	Standard

Media Supplements

MEM Amino Acids Solution

Cat. No.	5-12K01-H
Unit Size	100 ml
Presentation	50 × liquid
Formula	w/o Glutamine

Concentration	
Amino Acids	g/L
L-Arginine HCl	6.32
L-Cystine	1.20
L-Histidine HCl H ₂ O	2.10
L-Isoleucine	2.625
L-Leucine	2.62
L-Lysine HCl	3.625
L-Methionine	0.755
L-Phenylalanine	1.65
L-Threonine	2.38
L-Tryptophan	0.51
L-Tyrosine	1.80
L-Valine	2.34

MEM Non-Essential (NE) Amino Acids Solution

Cat. No.	5-13K00-H
Unit Size	100 ml
Presentation	100 × liquid
Formula	Standard

Concentration	
Amino Acids	g/L
L-Alanine	0.89
L-Asparagine H ₂ O	1.50
L-Aspartic Acid	1.33
L-Glutamic Acid	1.47
Glycine	0.75
L-Proline	1.15
L-Serine	1.05

L-Glutamine Solution

Cat. No.	5-10K00-H
Unit Size	100 ml
Presentation	100 × liquid
Formula	200 mM (28.2 g/L) L-Gln

L-Glutamine Powder

Cat. No.	5-10P00-O/-R
Unit Size	100 g/500 g
Presentation	powder

MEM Vitamins Solution

Cat. No.	5-21K00-H
Unit Size	100 ml
Presentation	100 × liquid
Formula	Standard

Concentration

Vitamins	g/L
Choline Chloride	0.10
D-Pantothenic Acid Ca Salt	0.10
Folic Acid	0.10
i-Inositol	0.20
Nicotinamide	0.10
Pyridoxal HCl	0.10
Riboflavin	0.01
Thiamine HCl	0.10

BME Amino Acids Solution

Cat. No.	5-11K01-H
Unit Size	100 ml
Presentation	50 × liquid
Formula	w/o Glutamine

Concentration

Amino Acids	g/L
L-Arginine HCl	1.052
L-Cystine	0.60
L-Histidine HCl H ₂ O	0.54
L-Isoleucine	1.30
L-Leucine	1.30
L-Lysine HCl	1.812
L-Methionine	0.375
L-Phenylalanine	0.825
L-Threonine	1.20
L-Tryptophan	0.20
L-Tyrosine	0.90
L-Valine	1.175

Stable Glutamine Solution

Cat. No.	5-10K50-H
Unit Size	100 ml
Presentation	100 × liquid
Formula	200 mM (43.44 g/L) L-Ala-L-Gln

Stable Glutamine Powder

Cat. No.	CN-38-O/-R
Unit Size	100 g/500 g
Presentation	powder

BME Vitamin Solution

Cat. No.	5-20K00-H
Unit Size	100 ml
Presentation	100 × liquid
Formula	Standard

Concentration

Components	g/L
Choline Chloride	0.10
D-Pantothenic Acid Ca Salt	0.10
Folic Acid	0.10
i-Inositol	0.20
Nicotinamide	0.10
Pyridoxal HCl	0.10
Riboflavin	0.01
Thiamine HCl	0.10
Biotin	0.10
NaCl	8.50

Diamond Vitamin Tween™ 80 Solution (40 x)

Cat. No.	5-78F00-I
Unit Size	500 ml
Presentation	40 x liquid
Concentration	
Components	mg/L
Menadion (Vitamin K3)	11
(+) -a-Tocopherolacetate	5
Ergocalciferol (Vit D2)	55
Vitamin A Acetate	55
DL-a-Lipoic Acid	6.7
D-Biotin	5.5
D-Calcium Pantothenate	5.5
Choline Chloride	276.7
Cyanocobalamin (Vitamin B12)	4
Folic Acid	5.5
i-Inositol	27.7
Niacin	10.6
Niacinamide	10.6
4-Aminobenzoic Acid (PABA, Vit H1) 27.7	27.7
Pyridoxal HCl	10.6
Pyrodoxin HCl	10.6
Riboflavin (Vitamin B2)	5.5
Thiamin HCl (Vitamin B1 HCl)	5.5
Tween™ 80	4445
Ethanol p.A.	70.06 ml

Tween™ is a registered trademark of CRODA International Plc.

Gelatin Type B From Bovine Skin

Cat. No.	5-76F00-H
Unit Size	100 ml
Presentation	liquid
Formula	2% solution

CHO Lipid Mix

Cat. No.	5-74S08-E/-H
Unit Size	20 ml / 100 ml
Presentation	liquid
Formula	1 000 x (Proprietary)

Lutrol® F-68

Cat. No.	5-75F02-H
Unit Size	100 ml
Presentation	liquid
Formula	10% solution

Transferrin (Human, holo)

Cat. No.	5-67F00-D/-G
Unit Size	100 ml / 50 ml
Presentation	liquid
Formula	4 mg/ml

BSA Solution

Cat. No.	5-66F00-H
Unit Size	100 ml
Presentation	liquid
Formula	100 mg/ml

2-Mercaptoethanol

Cat. No.	5-69F00-E
Unit Size	20 ml
Presentation	1 000 x liquid
Formula	50 mM

Nucleosid Mix (100 x)

Cat. No.	5-82F00-I/-K
Unit Size	500 ml / 1 L
Presentation	100 x liquid
Formula	Standard

Concentration

Components	mg/L
Cytidine	1 000
Guanosine	1 000
Uridine	1 000
Adenosine	1 000
2' Deoxyadenosine	1 000
2' Deoxycytidine HCl	1 100
2' Deoxyguanosine H ₂ O	1 067
2' Deoxythymidine	1 000

Sodium Pyruvate

Cat. No.	5-60F00-H
Unit Size	100 ml
Presentation	100 mM solution
Formula	11 g/L

Insulin (Human, Recombinant)

Cat. No.	5-79F00-G
Unit Size	50 ml
Presentation	liquid
Formula	5 mg/ml

Tryptose Phosphate Broth

Cat. No.	5-62F00-H
Unit Size	100 ml
Presentation	liquid
Formula	29.5 g/L

Phenol Red Solution

Cat. No.	5-70F01-H
Unit Size	100 ml
Presentation	liquid
Formula	1.5 g/L

HT-Supplement

Cat. No.	5-64K00-H
Unit Size	100 ml
Presentation	50 x liquid
Formula	Standard

Concentration

Components	g/L
Hypoxanthine	680
Thymidine	193
NaCl	9000

HAT-Supplement

Cat. No.	5-63K00-H
Unit Size	100 ml
Presentation	50 x liquid
Formula	Standard

Concentration

Components	mg/L
Hypoxanthine	680
Aminopterin	8
Thymidine	193
NaCl	9000

Glucose Solution (50%)

Cat. No.	5-33F01-H/-I
Unit Size	100 ml / 500 ml
Presentation	liquid
Formula	Standard

Concentration

Components	g/L
Glucose	500

Amino Acids CC grade (Powder)

Amino acid	Product Code (100g)	Product Code (500g)
L-Alanine	CN-9002-O	C-9001-R
L-Arginine	CN-9003-O	C-9003-R
L-Asparagine H ₂ O	CN-9006-O	C-9006-R
L-Aspartic Acid	CN-9007-O	C-9007-R
L-Cystine	CN-9009-O	C-9009-R
L-Glutamine	5-10P00-O	5-10P00-R
L-Glutamic Acid	CN-9013-O	C-9013-R
Glycin	CN-9014-O	C-9014-R
L-Histidine	CN-9015-O	C-9015-R
L-Isoleucine	CN-9019-O	C-9019-R
L-Leucine	CN-9020-O	C-9020-R
L-Lysine HCl	CN-9022-O	C-9022-R
L-Methionine	CN-9023-O	C-9023-R
L-Phenylalanine	CN-9024-O	C-9024-R
L-Proline	CN-9025-O	C-9025-R
L-Serine	CN-9026-O	C-9026-R
L-Threonine	CN-9027-O	C-9027-R
L-Tryptophan	CN-9029-O	C-9029-R
L-Tyrosine	CN-9030-O	C-9030-R
L-Valine	CN-9031-O	C-9031-R

Methylcellulose

Cat. No.	9-00F14-I/-K
Unit Size	500 ml / 1000 ml
Presentation	0.5% liquid
Formula	Endotoxin free, cell culture grade

Information

Methylcellulose is used as a carrier substrate for test substances that have to be injected into animals. Methylcellulose increases the viscosity of the cell culture medium and thereby protects the cell from mechanical stresses and fluid movements in the culture dish.

Trypan Blue

Cat. No.	5-72F00-H
Unit Size	100 ml
Presentation	0.5% liquid, not sterile
Formula	5 g/L in normal saline

Information

Trypan Blue is a stain recommended for use in dye exclusion procedures to count viable cells. Viable cells do not take up the dye whereas dead cells do.

Trypsin Solutions

Trypsin in PBS, with EDTA

Cat. No.	5-52F00-H/-I
Unit Size	100 ml / 500 ml
Presentation	1 × liquid
Formula	EDTA PBS 1:250 (0.25%/0.02%) w/o Ca ⁺⁺ /Mg ⁺⁺ w/o Phenol Red

Trypsin 0.25% in PBS, with EDTA, with Phenol Red

Cat. No.	5-54F00-H
Unit Size	100 ml
Presentation	10 × liquid
Formula	EDTA PBS 1:250 (0.25%/0.02%) with Phenol Red

Trypsin 0.5% in PBS, with EDTA

Cat. No.	5-51K00-H
Unit Size	100 ml
Presentation	10 × liquid
Formula	EDTA PBS 1:250 (10 ×) (0.5%/0.2%) with Phenol Red w/o Ca ²⁺ , Mg ²⁺

Trypsin 0.25% in PBS

Cat. No.	5-50F00-H
Unit Size	100 ml
Presentation	1 × liquid
Formula	0.25 % Trypsin in PBS w/o Ca ⁺⁺ /Mg ⁺⁺ with 10 mg/L Phenol Red

Trypsin 2.5% in PBS

Cat. No.	5-50K00-H
Unit Size	100 ml
Presentation	10 × liquid
Formula	2.5 % Trypsin in PBS w/o Ca ⁺⁺ /Mg ⁺⁺ with 100 mg/L Phenol Red w/o EDTA
Remarks	Dilute 1:10 with PBS w/o Ca ⁺⁺ /Mg ⁺⁺ (3-05F29) before use

Antibiotic / Antimycotic Solutions

PSN (Pen-Strep-Neomycin)

Cat. No.	4-13F00-H
Unit Size	100 ml
Presentation / ml	5 mg / 5 mg / 10 mg
Spectrum	Gram ⁺ / Gram ⁻

Geneticin® G418 50 mg/ml

Cat. No.	4-15F01-H/-E
Unit Size	100 ml / 20 ml
Used as a selective agent in mammalian cells at concentrations ranging from 400 to 1 000 µg/ml. G418 blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic cells. Resistance to G418 is conferred by the Neomycin resistance gene (neo) from Tn5 encoding an aminoglycoside 3'-phosphotransferase, APH(3')-II.	

Geneticin® G418 crystalline

Cat. No.	4-15P01-GA
Unit Size	5 mg
Used as a selective agent in mammalian cells at concentrations ranging from 400 to 1 000 µg/ml. G418 blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic cells. Resistance to G418 is conferred by the Neomycin resistance gene (neo) from Tn5 encoding an aminoglycoside 3'-phosphotransferase, APH(3')-II.	

Hygromycin B 50 mg/ml

Cat. No.	4-21F01-E
Unit Size	20 ml
Used as a selective agent in molecular genetics experiments on a wide variety of eukaryotic and prokaryotic species. The hph gene confers hygromycin-resistance to cells expressing it. Mammalian cells are sensitive to Hygromycin B concentrations of 50–200 µg/ml, and bacteria to 50–100 µg/ml.	

Amphotericin B (Fungizone®)

Cat. No.	4-05F00-H
Unit Size	100 ml
Presentation / ml	250 µg
Suggested Working Conc.	2.5 µg/ml
Spectrum	Fungi / Yeasts

Penicillin / Streptomycin / Fungizone®

Cat. No.	4-02F00-H
Unit Size	100 ml
Presentation/ml	10000 IU/10000 µg/25 µg
Suggested Working Conc.	1:100
Spectrum	Bacteria, Fungi, Yeasts

Penicillin

Cat. No.	4-12F00-H
Unit Size	100 ml
Presentation/ml	10000 IU
Suggested Working Conc.	1:100
Spectrum	Gram ⁺

Penicillin / Streptomycin

Cat. No.	4-01F00-H
Unit Size	100 ml
Presentation/ml	10000 IU/10000 µg
Suggested Working Conc.	1:100
Spectrum	Gram ⁺ / Gram ⁻

Gentamicin

Cat. No.	4-07F00-H
Unit Size	100 ml
Presentation/ml	5000 µg
Suggested Working Conc.	50 µg/ml
Spectrum	Gram ⁺ / Gram ⁻ / Mycoplasma

Neomycin

Cat. No.	4-10F00-H
Unit Size	100 ml
Presentation/ml	10000 µg
Suggested Working Conc.	50 µg/ml
Spectrum	Gram ⁺ / Gram ⁻

Chlortetracyclin

Cat. No.	4-06F00-H
Unit Size	100 ml
Presentation/ml	5000 µg
Suggested Working Conc.	20 µg/ml
Spectrum	Gram ⁺ / Gram ⁻

Tylosin

Cat. No.	4-04F00-H
Unit Size	100 ml
Presentation/ml	5000 µg
Suggested Working Conc.	1:100–1:50
Spectrum	Gram ⁺ / Gram ⁻ / Mycoplasma

Kanamycin

Cat. No.	4-08F00-H
Unit Size	100 ml
Presentation/ml	5000 µg
Suggested Working Conc.	50–100 µg/ml
Spectrum	Gram ⁺ / Gram ⁻ / Mycoplasma



Sera

Fetal Calf Sera (FCS)

Newborn Calf Sera (NCS)

Calf Sera (CS)

Newborn Horse Sera (NHS)

Horse Sera (HS)

Other Sera

Seahorse [*Hippocampus*]

Seahorse is the name given to over 40 species of small marine fishes in the genus *Hippocampus*. "Hippocampus" comes from the Ancient Greek *hippokampos*, itself from *hippos* meaning "horse" and *kampos* meaning "sea monster". They are named for their equine appearance with bent necks and long snouted heads followed by their distinctive trunk and tail. Seahorses swim upright in a unique way, rapidly fluttering a dorsal fin and using pectoral fins to steer. They are adept at camouflage with the ability to grow and reabsorb spiny appendages depending on their habitat.



Sera

Serum Types

Charcoal Stripped Sera

BioConcept offers charcoal/dextran stripped sera for researchers requiring low levels of hormones. Charcoal/dextran stripping reduces the concentration of steroid hormones in sera such as estradiol, progesterone, cortisol, testosterone, T3 and T4. These sera are useful where endogenous molecules may interfere with experimental works.

Gamma Irradiated Sera

BioConcept Ltd. offers Gamma-irradiated sera. Gamma-irradiation is performed at 25 kGy. Gamma irradiated sera minimize the risk associated with the use of animal products and offers protection against low levels of microbial contaminants. The treatment inactivates possible viruses while maintaining growth promotion potential.

Dialyzed Sera

BioConcept Ltd. offers dialyzed sera. Dialysis reduces the concentration of free low molecular weight components such as nucleotides, amino acids, hormones and ions. A dynamic filtration method is used to produce dialyzed sera.

Tetracycline Free Sera

BioConcept Ltd. offers tetracycline free sera. Sera are tested for the presence of chlortetracycline, oxytetracycline and tetracycline by a liquid chromatography electro spray ionisation tandem mass spectrometry method. The detection limit is < 0.05 mg/l.

BioConcept Ltd.'s Sampling Policy

BioConcept Ltd. provides free samples of up to three different batches combined with a batch reservation. Minimum batch reservation is ten 500 ml bottles. These batches are reserved for our customers for a test period of up to 30 days, and of up to 60 days if needed. The sample size is 50 ml per batch. If needed, we can adapt this general policy to the needs of our customers (please request).

After batch testing BioConcept Ltd. can store prepaid reservations up to 24 months in our -20°C storage rooms (free of charge) for customers who do not have enough storage facilities. Customers can call off their prepaid sera according to their demands.

Quality Control

Virus Testing

All calf sera are tested for adventitious viruses using cell culture techniques.

Test include:

- Bovine Viral Diarrhoea (BVD)
- Infectious Bovine Rhinotracheitis (IBR)
- Parainfluenza Type 3 (PI3)

All sera are tested for the absence of the indicated viruses by inoculation with GBK cells. The revelation is made by indirect immunofluorescence. Antibody testing: presence of specific antibodies is detected utilizing an elisa assay.

Mycoplasma

Each final product batch is tested for the absence of mycoplasma. All sera are tested for the absence of Mycoplasma utilizing a 21 days cell culture assay by the culture method.

Physical Parameters – Osmolality and pH

Osmolality is determined by the lowered freezing temperature. Osmometer and pH-meters are daily calibrated using standard solutions.

Endotoxins

All sera are tested to determine the levels of endotoxins. Two tests are performed: turbidimetric, a qualitative test, and chromo-kinetic, a quantitative test. The endotoxin reagent is standardized against the US reference endotoxin. The method is based on “the bacterial endotoxins test” USP.

Cell Culture Testing

Each final batch of serum is tested for its ability to support in vitro growth of specific cell lines. Therefore, in addition to verify that each batch of serum passes quality control specifications, three important performance criteria are evaluated, these are: Growth Promotion, Cloning Efficiency, Plating Efficiency. Following cell lines are used: L929, Hela, MRC-5 and Sp2/O-Ag14.

Heat Inactivated Sera

BioConcept Ltd. offers heat inactivated sera. Heat inactivation is performed at 56 °C for 30 minutes. Heat inactivation destroys the complement which is needed especially in some immunology experiments. Heat inactivation can also inactivate viruses and bacterial contaminants such as mycoplasma.

Geographical BSE Risk Classification (GBR)

BioConcept Ltd. supplies a wide range of sera origins including sources from South America as well as from the European Union (EU) and from sources approved by United States Department of Agriculture (USDA).

The choice of FBS origin is determined by customer needs, import requirements and worldwide supply.

BioConcept Ltd. is the ideal partner for meeting different requirements, such as choosing the origin of serum, which provides optimal performance and results.

FBS is considered to be an animal by-product which is not intended for human consumption.

Biological safety is controlled by EU rules on animal by-products:

- Regulation EU 1069/2009 and regulation EU 142/2011
- Official disease status BSE, FMD, etc.

Individual countries are monitored for their animal health status by the World Organisation for Animal Health (OIE)

More information:

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003700.pdf

Fetal Calf Sera (FCS)

Product No.	Unit Size	Presentation	Origin
2-01F10-I	500 ml	Standard	South America EC approved
2-01F16-I	500 ml	Heat Inactivated	South America EC approved
2-01F17-I	500 ml	Dialyzed	South America EC approved
2-01F00-I	500 ml	Standard	Europe EC approved
2-01F26-I	500 ml	Heat Inactivated	Europe EC approved
2-01F28-I	500 ml	Tetracycline free	Europe EC approved
2-01F29-H	100 ml	Charcoal stripped	Europe EC approved
2-01F29-I	500 ml	Charcoal stripped	Europe EC approved
2-01F30-I	500 ml	Standard	Brasil
2-01F36-I	500 ml	Heat Inactivated	Brasil
2-01F40-I	500 ml	Standard	USA
2-01F46-I	500 ml	Heat Inactivated	USA
2-01F80-I	500 ml	Standard	Australia
2-01F86-I	500 ml	Heat Inactivated	Australia
2-01F90-I	500 ml	Standard	New Zealand
2-01F96-I	500 ml	Heat Inactivated	New Zealand
2-01F120-I	500 ml	Standard	Mexico
2-01F126-I	500 ml	Heat inactivated	Mexico

Newborn Calf Sera (NCS)

Product No.	Unit Size	Presentation	Origin
2-02F110-I	500 ml	Standard	Costa Rica
2-02F80-I	500 ml	Standard	Australia
2-02F86-I	500 ml	Heat Inactivated	Australia

Calf Sera (CS)

Product No.	Unit Size	Presentation	Origin
2-03F00-I	500 ml	Standard	Europe

Newborn Horse Sera (NHS)

Product No.	Unit Size	Presentation
2-04F00-I	500 ml	Standard

Horse Sera (HS)

Product No.	Unit Size	Presentation
2-05F00-I	500 ml	Standard
2-05F26-I	500 ml	Heat Inactivated

Other Sera

Product No.	Unit Size	Presentation
2-11F00-H	100 ml	Mouse Serum
2-12F00-H	100 ml	Rat Serum
2-13F00-H	100 ml	Human Serum
2-14F00-H	100 ml	Pig Serum
2-14F00-I	500 ml	Pig Serum
2-15F00-H	100 ml	Sheep Serum
2-15F00-I	500 ml	Sheep Serum
2-16F00-H	100 ml	Goat Serum
2-16F00-I	500 ml	Goat Serum
2-17F00-H	100 ml	Chicken Serum
2-17F00-I	500 ml	Chicken Serum

If you need sera not described please contact us. We can also supply you with other types of sera not mentioned in these lists.



Technical Information / Protocols

Guide to Supplementation of Media and Media handling

Standard Packaging and Flexibility

Protocols

Christmas tree worm [*Spirobranchus giganteus*]

The Christmas tree worm, named after its unique structure, can be found throughout the world's tropical oceans. The worm lives within its tree-like features which are used to capture prey and transport it to the worm's mouth.

They also help the worm breathe by increasing the radius of respiration. This is why the structures are quite often referred to as gills. In fact, its Latin name, *spirobranchus*, essentially translates to 'spiral gills'. The part we can see of the worm is actually only about one third of it, the other two thirds live within the rock it is attached to.



Technical Information

BioConcept Ltd.'s raw material and components are of the highest quality available. They are only purchased from approved and ISO-certified suppliers. All chemicals can be traced back to the lots used. During the manufacturing process the weights of the chemicals are controlled automatically by computerized balances. This guarantees a high degree of reproducibility of the formula. BioConcept Ltd.'s water system comes from the Swiss market leader in pure water technology. With our facilities we can produce different levels of purified water, from standard purified water to WFI. This means we can offer ultra pure, apyrogenic water which is compliant to USP standards.

The sizes of the batches produced start from 5 L, giving BioConcept Ltd. the flexibility to better serve its customers.

In BioConcept Ltd.'s quality control department, all liquid media are routinely tested for sterility, growth promotion and absence of cytotoxicity, the osmolality required and the correct pH level. Powdered media are reconstituted with cell culture grade water and checked as described for liquid media.

Guide to Supplementation of Media and Media handling

Unless noted otherwise, all 1 L liquid media are formulated without antibiotics, serum and L-Glutamine as Glutamine is the most unstable component in liquid cell culture media. 10× concentrated media do not contain antibiotics, serum, L-Glutamine and sodium bicarbonate. Powder media contain L-Glutamine but no sodium bicarbonate. We recommend aseptically adding the required amount of these supplements prior to use. For the addition of the appropriate amounts of L-Glutamine and sodium bicarbonate, please refer to Table 1 below for the addition of antibiotics, please refer to Table 3 on **page 105**. Sodium bicarbonate can be added either as a solid before sterilization or as a 7.5% sterile solution after sterilization. Serum is usually added to the cell culture media to a final concentration of 1 to 25%.

Table 1: Guide to the addition of L-Glutamine (200 mM solution)

Medium (1 L)	L-Glutamine (200 mM/l) ml required	Final concentration of L-Glutamine (mg/L)
DMEM	20.0	584
DMEM/Ham F12 Standard	20.0	365
Ham's F-10	5.0	146
Ham's F-12	5.0	146
MEM Iscove's	20.0	584
Leibovitz L-15	10.3	300
M199 Earle's	3.4	100
M199 Hank's	3.4	100
McCoy's 5A	7.5	219
MEM Alpha	10.0	292
MEM Earle's	10.0	292
MEM Hank's	10.0	292
RPMI 1640	10.3	300
Waymouth's	12.0	350
William's E	10.0	292

Table 2: Guide to the addition of Sodium Bicarbonate (7.5% solution or powder)

Medium (1 L)	NaHCO ₃ (7.5% solution) ml required	NaHCO ₃ (solid) g required	Final concentration of NaHCO ₃ (mg/L)
DMEM	49.3	3.70	3700
DMEM/Ham F12 Standard	32.5	2.438	2438
Ham's F-10	16.0	1.20	1200
Ham's F-12	15.7	1.176	1176
MEM Iscove's	40.4	3.024	3024
Leibovitz L-15	–	–	–
M199 Earle's	29.3	2.20	2200
M199 Hank's	4.7	0.35	350
McCoy's 5A	29.3	2.20	2200
MEM Alpha	29.3	2.20	2200
MEM Earle's	29.3	2.20	2200
MEM Hank's	4.7	0.35	350
RPMI 1640	26.7	2.00	2000
Waymouth's	29.9	2.24	2240
William's E	29.3	2.20	2200

Preparation of 1 L Single Strength Media from 10× Concentrate

1. Aseptically measure out approximately 850 ml of sterile tissue culture grade water in a container of appropriate size.
2. Gently stir and aseptically add 100 ml of 10× medium.
3. Now add the required amount of Sodium Bicarbonate.
4. Sera, antibiotics, L-Glutamine and other supplements may now be added to the solution.
5. Use sterile 1 N NaOH or 1 N HCl to adjust the pH of the solution to the desired value.
6. Use tissue grade water to adjust the final volume of the solution to 1 L.
7. The completed medium has a limited shelf life and should be refrigerated.

Preparation of 1 L Liquid Media From Powder Media

1. Place approximately 900 ml of cell culture water in a 1 L Erlenmeyer flask. While gently stirring, slowly add the powder. Do not heat.
2. Add solid Sodium Bicarbonate. Stir and adjust the solution with water to 1 L.
3. Control the pH and, if necessary, adjust with 1 N HCl to approximately 0.2 pH units below the value required after sterilization.
4. Use 0.2 µm filter to sterilize the medium.

Storage Conditions and Shelf Life of Media

Storage conditions and shelf life of media are indicated on the label.

Powder Media Use

Since it has been demonstrated that powdered media can be prepared by milling of the component chemicals, the use of powdered media has grown significantly. The use of a powdered medium allows the investigator uniformity that is not usually attainable from conventionally prepared liquid media. This is because a single batch of medium may be employed in long term or replicate experiments. Long term stability of powders and easy storage are two of the advantages of powdered media often cited. Autoclavable powdered media have not been employed routinely although the usefulness of such preparations is readily apparent. Succinic acid and sodium succinate are utilized to maintain the pH of 4.0–4.5 during autoclaving.

Media with Stable Glutamine

In liquid solutions, L-Glutamine is not stable at temperatures of 4 °C or higher. The molecule dissociates and creates toxic ammonium as a waste product of that reaction. This restricts the stability of cell culture media and as a consequence, stock solutions of L-Glutamine or solutions containing Glutamine have to be stored frozen. Additionally, cell culture media have to be changed frequently to ensure the availability of the amino acid.

Dipeptides containing Glutamine are extremely well suited as a replacement of L-Glutamine. They are stable over long periods of time, even if stored at room temperature. Cells may recover L-Glutamine via cleavage of the peptide bond using cellular peptidases. BioConcept Ltd. offers several media with stable Glutamine in the form of L-Ala-L-Gln (Cat. No: 5-10K50-H). The amount of available L-Glutamine corresponds to that of L-Glutamine normally present in the equivalent medium after the addition of the appropriate amount of L-Glutamine.

Media with HEPES

If you have a sensitive cell culture system, we recommend that you use HEPES buffered media. Such nutrient solutions are prepared in accordance with the original formulation and include Sodium Bicarbonate. However, to maintain an appropriate osmolality, the concentration of Sodium Chloride is decreased. HEPES increases the buffering capacity and the stability of the pH in the range of 7.2 to 7.6.

This allows the media to better resist fluctuations in the pH resulting from changes of cellular metabolism especially in rapidly growing cultures with a strong acidulation. BioConcept Ltd. offers several media with HEPES (see section Special Media).

Media without Phenol Red

Phenol Red is not a natural substance. Nevertheless, it is added as an indicator of the pH to cell culture media. However, the presence of this dye is not always desired. Especially in the cultivation of primary cells, media with Phenol Red may produce artifacts. Additionally, Phenol Red may interfere with chromogenic bioassays. BioConcept Ltd. offers several media without Phenol Red, if you cannot find your media, please contact BioConcept Ltd. using the information at the back of this catalogue.

Deficient Media

Deficient MEM media are suited for radioactive incorporation studies to investigate metabolic aspects of cellular physiology. BioConcept Ltd. offers several deficient MEM-media. We can produce any deficient media, including labelling WMR.

Serum-Free and Protein-Free Media

Normally, serum has to be added to cell culture media. It has multiple functions, for example, it may serve as a source of nutrients, enzymes, hormones and growth factors; it may also serve as a pH-buffer or may bind toxic components. However, serum has quite a lot of disadvantages; it is expensive, shows lot-to-lot-variability and increases the complexity of the down-stream processing of the desired biological material. BioConcept Ltd. offers several serum-free and protein-free media.

Preparation of Customer-Designed Media

We make specialized modification to standard cell culture media as well as nutrient mixtures that are adapted to cell requirements. In our modern manufacturing plant we produce under strictly controlled conditions to create formulations. Our employees have many years of experience in the production of cell culture media and we have extensive in-process and end-controls to ensure that your product is of the utmost quality.

If you want to order your special media, please e-mail us your formulation at info@bioconcept.ch. If you have additional questions on your special media please do not hesitate to contact us!

If you are interested, BioConcept Ltd. would like to show you its production plant in Allschwil, Switzerland. If you would like to know how we manufacture your cell culture media, please contact your local BioConcept Ltd. representative or directly call + 41 (0)61 486 80 80.

Serum Handling Tips

At BioConcept Ltd., our goal is to provide maximum value with our products to all of our customers. To help ensure that you get satisfactory performance from your order of BioConcept Ltd. serum, BioConcept Ltd. recommends that you adhere to the following handling guidelines.

BioConcept Ltd. serum should be inspected on arrival and stored at –20 °C. Should the serum become partially thawed, we recommend that you leave it to thaw completely at room temperature and then carefully mix, avoiding frothing. The serum may be refrozen and stored at –20 °C before use. In our experience, this will not decrease the growth promoting properties of the serum, and will help prevent precipitates and complexes from forming in the serum. Avoid glass-to-glass and glass-to-metal contact. Most glass breakage is caused by bruising resulting from this type of contact. Avoid rapid temperature changes. Never transfer a glass bottle directly from a freezer to a water bath. Conversely, avoid transferring glass bottles directly from a water bath to a freezer. Always keep some space between serum bottles during storage.

Thawing Serum: When serum has been stored at –20 °C, it should be allowed to equilibrate in a refrigerator at 2–8 °C (preferably overnight). Placing a piece of cloth under the bottles during equilibration helps reduce glass breakage. Allow thawing

to complete at room temperature. Thawing serum at high temperatures is not recommended. Periodic agitation during thawing is helpful. Turbidity or flocculent material may appear upon thawing or storage. This is caused by lipoproteins that may precipitate. This will not adversely affect the performance of the product. Complement is inherent in sera and can be inactivated by raising the temperature to 56 °C (heat inactivation).

Heat Inactivation: BioConcept Ltd. offers FCS in a heat inactivated form upon request. However, it is a rather simple procedure to inactivate serum. Essentially, all that is needed to be done is to incubate the serum at 56 °C in a water bath for 30 minutes. The serum must be gently swirled every 10 minutes during this incubation. Please be advised that heat inactivation may cause serum gelation. Sera with higher protein concentrations will gel more easily. Also, heat inactivation at higher temperatures or for longer periods of time may promote gelation.

Using Antibiotic/Antimycotic solutions

The following table is a general guide for usage of antibiotics in cell culture media containing serum. Serum-free media require lower concentrations and should be determined empirically. Stability of these antibiotics in cell culture media is at least 3 days at 37 °C.

Table 3: Guide for the addition of antibiotics
Fungizone is a registered trade mark of E.R. Suibb & Sons

Cat. No.	Product Description	Presentation/ml	Suggested Working Concentration	Spectrum
4-01F00-H	Penicillin/ Streptomycin	10 000 IU/ 10 000 µg	1:100	Gram ⁺ , Gram ²
4-02F00-H	Penicillin/ Streptomycin/ Fungizone®	10 000 IU/ 10 000 µg/25 µg	1:100	Bacteria, Fungi, Yeasts
4-03F00-H	Penicillin/ Streptomycin/ Fungizone®	10 000 IU/ 10 000 µg/250 µg	1:100	Bacteria, Fungi, Yeasts
4-05F00-H	Amphotericin B/ Fungizone®	250 µg	1:100	Fungi, Yeasts
4-07F00-H	Gentamicin	5 000 µg	1:100	Gram ⁺ , Gram ² , Mycoplasma
4-10F00-H	Neomycin	10 000 µg	1:200	Gram ⁺ /Gram ²
4-15F01-H	G418/Geneticin®			
	Used as a selective agent in mammalian cells at concentrations ranging from 400 to 1 000 µg/ml. G418 blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic cells. Resistance to G418 is conferred by the Neomycin resistance gene (neo) from Tn5 encoding an aminoglycoside 3'-phosphotransferase, APH 3' II.			
4-15P01-GA	G418/Geneticin® crystalline			
	Used as a selective agent in mammalian cells at concentrations ranging from 400 to 1 000 µg/ml. G418 blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic cells. Resistance to G418 is conferred by the Neomycin resistance gene (neo) from Tn5 encoding an aminoglycoside 3'-phosphotransferase, APH 3' II.			
4-21F01-E	Hygromycin B			
	Used as a selective agent in molecular genetics experiments on a wide variety of eukaryotic and prokaryotic species. The hph gene confers hygromycin-resistance to cells expressing it. Mammalian cells are sensitive to Hygromycin B concentrations of 50–200 µg/ml, and bacteria to 50–100 µg/ml.			

Biological Buffers and pH Control

All media used in tissue culture have a synthetic mixture of inorganic salts known as a 'physiological' or balanced salt solution. The basic functions of this salt solution in the medium are to maintain proper pH, maintain ideal osmotic pressure, and provide a source of energy. The growth of animal cells in a nutritionally complete tissue culture medium is usually optimal when the medium is buffered at a pH in the range of 7.2–7.4. To function most effectively, the pKa of the chosen buffer should be as close as possible to the required pH.

The most commonly used buffer in tissue culture media is sodium bicarbonate. However, this buffer has two important disadvantages:

1. The pKa of sodium bicarbonate is 6.3 at 37 °C which results in suboptimal buffering throughout the physiological pH range, and
2. Carbon dioxide is released in the atmosphere which results in an increase in alkalinity, and the number of hydroxyl ions produced increases according to the amount of sodium bicarbonate added to the medium. It is possible to control this by artificially supplying carbon dioxide to the atmosphere and preventing the gas from leaving the liquid, thereby reducing the hydroxyl ion concentration in solution.

Balanced salt solutions can be divided into two types: those intended to equilibrate with air in a closed system at a low concentration of sodium bicarbonate (Hank's Balanced Salt Solution) and those intended to equilibrate with a gaseous phase containing approximately 5% CO₂ at a higher concentration of sodium bicarbonate (Earle's Balanced Salt Solution Cat. No: 3-01F00-I). Earle's Balanced Salt Solution is a much better solution because it contains a greater amount of sodium bicarbonate, but it is more difficult to use since it requires a special gaseous mixture of 5% CO₂ with 95% air to be provided by the culture medium. If this procedure is not carried out, the pH increases rapidly at normal incubation temperatures. The purple color of the medium indicates that the pH has risen, and cell growth is inhibited. An alternative method is to use a medium which produces sufficient buffering capacity but does not require 5% CO₂.

In some cases, this can be achieved by using a medium containing Earle's salts but having the concentration of sodium bicarbonate reduced to 0.85 g/liter. An entirely different approach was devised by Leibovitz (1963). He utilized the buffering capacity of free base amino acids, omitted sodium bi-carbonate, substituted galactose for glucose and added pyruvate. The pH of his L-15 medium is approximately 7.8 which is higher than that of most other media. Since there is no production of loss of CO₂, the pH will not rise further. This medium makes possible the growth of cells in open culture vessels without regard to the CO₂ content of the atmosphere.

Attempts have been made in recent years to find the most suitable buffer. The most commonly utilized alternative to bi-carbonate is HEPES buffer which was first described by Good, et al. (1966). It acts as a zwitterion and has proved superior to conventional buffers in comparative biological such as with cell-free preparations. It has many properties which make it ideal as a buffer to tissue culture media, principally in that it does not require an enriched atmosphere to maintain the correct pH. HEPES does not bind divalent cat ions and is soluble to the extent of 2.25 M at 0 °C. **Note:** since the DpKa/°C of 20.014, the pH reading recorded in a HEPES buffered medium will vary inversely with the temperature of the medium.

Expected pH levels at various temperatures

°C	pH	°C	pH
5	7.58	23	7.47
15	7.56	24	7.46
16	7.55	25	7.44
17	7.54	26	7.43
18	7.53	27	7.42
19	7.52	28	7.41
20	7.50	29	7.40
21	7.44	30	7.38
22	7.48	37	7.30

HEPES (Cat. No: 5-13F00-H) is satisfactory as a buffer in tissue culture media for the growth of many different types of cells and viruses. It may exhibit toxicity at concentrations greater than 40 mM. Studies have indicated that 20 mM HEPES is the most satisfactory concentration for the buffer when both Hank's and Earle's solutions are used. CO₂ incubators should not be used with media buffered solely with HEPES. BioConcept Ltd. HEPES buffered liquid media are produced with a pH of 7.2–7.4 at 37 °C. Powdered media are prepared so that a 1 L 3 solution will have a pH of 7.2–7.4 at 37 °C without further adjustment.

Sodium bicarbonate should also be added as a nutritional requirement. It is recommended that the sodium bicarbonate concentration should not exceed 10 mM when the HEPES concentration is 20 mM. All single-strength (1 L) liquid media contain either sodium bicarbonate or HEPES buffer or both. 10× concentrated (10 L3) liquid media do not contain buffer and powdered media are either buffer free or contain HEPES buffer only. Whenever sodium bicarbonate is used to buffer tissue culture media at a concentration of greater than 1.0 g/L, a CO₂ enriched atmosphere is required, which can be created through using a CO₂ incubator.

Mammalian cells can survive over a wide pH range 6.6–7.8, but as a rule, the optimal growth of cells is obtained at pH 7.2–7.4. It is undesirable to allow the pH to deviate outside the limits of pH 6.8–7.6. It should be remembered that no buffer is capable of holding the pH constant in a system in which acids or bases are being produced. Buffers only slow the rate of pH change. Cells in culture produce acidic products which act to lower the pH of the medium. Most of the media utilize phosphates and the bicarbonate system to buffer the media. The bicarbonate ion can be converted to gaseous carbon dioxide and lost from the medium resulting in a rise in the pH. Carbon dioxide can be maintained by supplying a special gas phase or by sealing the vessel tightly so that the CO₂ produced by metabolic processes is retained in the vessel and re-absorbed by the medium. Efforts to eliminate bicarbonate as a buffer system have been reported in medium SR1-8 and in medium L-15. Both media contain a phosphate buffer and increased amounts of amino acids to accomplish the buffering required.

A number of organic compounds have been described that have buffering capacity in the required range. HEPES will have a pronounced effect on the final pH. It is necessary to measure the pH at the temperature of use to determine the final pH due to the contribution by other buffers. HEPES buffer may be sterilized by steam and adjusted to desired pH with sodium hydroxide. Concentrations of 10–25 mM have been employed with no apparent toxicity. When utilizing HEPES, BioConcept Ltd.

chooses a 15–25 mM concentration in the preparation of media unless it is otherwise specified. A mixture of acid form and base form HEPES is used in combination in most of BioConcept Ltd.'s media at this has been found to produce a more stable buffering system. Sodium chloride is reduced to keep the osmolality of the media containing both bicarbonate and HEPES in the range on non-HEPES containing media.

Aseptic Techniques

70% of all problems are due to a lack of sound aseptic technique. Microorganisms which can result in contamination exist everywhere, from the surfaces of objects to the surrounding air. A conscious effort must be made to keep them out of the sterile environment. Because cell culture techniques often entail several steps which can lead to contamination, cell culture media are often supplemented with antibiotics. Purchasing cell culture media from premium vendors such as BioConcept Ltd. where quality assurance measures are rigorous will also greatly reduce the incidence of contamination. Nevertheless, this will not eliminate cell culture contamination resulting from poor sterile technique or antibiotic resistant mutants. Autoclaving will render pipettes, glassware and solutions sterile. Nutrient media cannot be autoclaved. The compounds in nutrient media are destroyed by the heat generated during autoclaving. Media must therefore be sterilized by sterile filtration through filters small enough in pore size to hold back bacteria and mycoplasma.

Guidelines for Sterile Techniques

1. Wipe your work area clean with 70% ethanol which has been sterile filtered prior to use. Filtration is required as 70% ethanol can be a good preservative for Fungi spores (Cat. No: 10-03F12-I). It may help to rinse your hands with ethanol as well.
2. Keep sterile flasks, bottles, and petri-dishes covered until the instant of use. Return any covers as soon as you are finished.
3. Sterile pipettes should NEVER be taken from the cylinder or wrapping until they are ready to be used. Keep your pipettes at your work area. Sterile pipettes DO NOT have to be flamed. Pipetting your cells with a hot pipette will kill them.
4. When removing the cap from a bottle, flask, etc., keep it face down, grasping it with the little finger of your right hand. DO NOT place caps on the lab bench. Flame the lips of flasks, bottles and other culture ware before and after introduction of a pipette. DO NOT hold the opening straight into the air. If possible, tilt the container such that any falling microorganisms will land in the lip where they will be flamed upon closure.
5. Avoid talking, singing or whistling while performing sterile procedures as these generate aerosol contaminants.
6. NEVER PIPETTE BY MOUTH. Use a pi-pump or other device. Even though your pipettes are plugged, mycoplasma in your mouth can still pass through and become one of the largest problems in cell culture contamination.
7. DO NOT draw from a bottle more than once with the same pipette. Use a sterile pipette each and every time – especially when pipetting media!
8. Techniques should be performed as rapidly as possible to minimize contamination. It is possible that you may find yourself performing a procedure not addressed by these sterile technique guidelines. Therefore, you must constantly be aware that microorganisms are everywhere and take proper steps to keep them out of your cultures. When first developing your aseptic technique, you must always think of sterility. Eventually, it will become second nature to you. Mastering good aseptic technique will save minimization of biohazard risk when infectious organisms or dangerous chemicals are being used.

Adaptation of Cells to Serum

The majority of established cell types currently cultured in media supplemented with FCS can be adapted to grow with BioConcept Ltd. Calf, Donor Calf or New-born Bovine Serum. Complete adaptation may take between three to four subcultures and should be carried out using a 10% serum concentration. Cells growing in smaller serum concentrations should be brought back to 10% serum before adaptation is attempted. The following method is a basic guide to the procedure:

1. Two days prior to sub culturing, nourish the cell line with defined medium containing 5% FCS and 5% alternative serum (known as “adaptation serum”).
2. 48 hours afterward, trypsinize the cells and split them using “adaptation medium”.
3. Nourish the cells with “adaptation medium” until confluent.
4. Repeat steps 2 and 3 for 1–2 subcultures.
5. Split the confluent cells using media containing 10 % alternative serum.
Note: Most heteroploid lines can be grown on a variety of sera without affecting the growth rate. Diploid lines are more fastidious but can be adapted. Primary cell cultures should be initiated on newborn calf serum wherever possible.

Eye Examination of Cell Cultures

Before commencing the general health of any culture should be evaluated. This can be done quickly and quantitatively by making the following observations.

1. Check the pH of the culture by examining the color. As a culture becomes more acidic, the indicator (phenol red) shifts from red to yellow. In contrast, as a culture becomes more alkaline, its color shifts from red to purple. In general, cells can tolerate slight acidity better than slight basicity. Any shift above pH 7.6 is particularly detrimental. Until you become familiar with the color of the indicator at various pH levels, use color standards for comparison.
2. Cell attachment of monolayer cultures should be to the well and spread out evenly. If cells are floating in the culture, determine if they are in a state of division or if they are dying. A dying cell will have an irregular morphology (alternatively, you can test for cell viability using Trypan Blue from BioConcept Ltd. Cat. No: 5-72F00-H).
3. The “percent confluency” or growth rate of a culture can be estimated by following it toward the development of a full cell sheet (i.e. a “confluent culture”). By comparing the amount of space covered by cells with the unoccupied spaces you can estimate the percent confluency.
4. The cell shape is an important guide. Round cells in an uncrowded culture are not a good indicator unless these happen to be dividing cells. Look for doublets or dividing cells. Get to know the effect of crowding on cell shape.
5. Look for “giant cells”. The number of “giant cells” will increase as a culture ages or declines in “well-being”. The frequency of “giant cells” should be relatively low and constant under uniform culture conditions.
6. One of the most valuable early indicators in assessing the success of a “culture split” is the rate at which the cells in the newly established cultures attach and spread out. Attachment within an hour or two suggests that the cells have not been traumatized and that the in vitro environment is not grossly abnormal. Longer attachment times are suggestive of problems. Nevertheless, good cultures may result even if attachment does not occur for four hours.
7. Learn to recognize the range of cell shapes and growth patterns exhibited by each cell line. For example, many transformed cells will “pile up” due to a lack of contact inhibition. This effect becomes more pronounced as the culture becomes overcrowded.

Mycoplasma Contamination

Mycoplasma are prokaryotic cellular organisms and are the smallest cells capable of autonomous growth. They lack a rigid cell wall and are pleomorphic. Additionally, they are sensitive to oxygen tension and osmotic shock. Many of the smaller mycoplasma can pass through bacterial filters. The cell size ranges from 0.15 to 1.0 μm . Mycoplasma can cause economically important infections of the respiratory tract, mammary glands, genital tracts or synovia of cattle, sheep, goats, cats, mice, rats, pigs and poultry. Some effects of mycoplasma infection on cell culture have been reviewed (McGarrity, et al., 1984). These infections DO NOT always result in microscopic alterations of cells or media. Many infections grow slowly and do not de-story host cells but can still alter the metabolism of the culture in subtle ways. The exact manner in which contamination of cell cultures occurs may be impossible to ascertain in any given instance. Studies by Barile and others (Barile, et al., 1978) have demonstrated that mycoplasma infection or contamination of cultures is the result of a number of factors, involving the investigator, the type of cell culture used and the media used in handling the cells. McGarrity (1976) concluded that mycoplasma-infected cultures are the most common source of further contamination. He listed recommendations for prevention and control of mycoplasma infection. These can be summarized as the following combination of practices:

- The use of primary cultures rather than propagated cell types.
- The use of heat-inactivated serum.
- The avoidance of direct mouth pipetting.
- The strict enforcement of good aseptic techniques.
- The use of antibiotic free media.
- The use of protective clothing by technicians.
- The use of premium cell biology vendors such as BioConcept Ltd. where maximum QC measures are employed.
- The use of prophylactic tools.
- The use of high concentration of gentamicin can reduce Mycoplasma contamination.

Subculturing Protocol Anchorage-Dependent Cells (Short Method)

1. Decant supernatant fluid from the culture plate into a waste collection jar, taking care to use sterile technique.
2. Add 3 ml of cold trypsin (Cat. No: 5-51F00-H) to the culture flask.
3. Incubate for 30 seconds (or longer if necessary). Examine at low magnification. When it appears that some of the cells have rounded up, but have yet to detach, decant the trypsin into a waste container. Continue to incubate the flask until virtually all cells have rounded up and can be readily dislodged.
4. Using a 10 ml pipette, add 10 ml of fresh MEM (Cat. No: 1-31F01-l) to the T-flask. Dispensing the MEM as a strong stream will aid in dislodging the cells.
Note: 10 ml is sufficient for a 1:2 split. Next, using the same pipette, draw up the cell suspension and quickly dispense a 5 ml aliquot into two 25 cm² T-flasks (one new, one old).
5. Incubate and monitor the flasks periodically beginning 30–45 minutes after inoculation. Rapid attachment (usually within 1 hour) indicates that the split has been successful.
6. Continue to monitor the culture's progress.

Always employ sterile techniques throughout. The above volumes correspond to a 25 cm² T-flask.

Haemocytometer Counting and Cell Viability

Accurate enumeration of cell density is an important aspect of cell culture. Cell enumeration with a haemocytometer is the most widely used method, and it continues to be used in most cell culture laboratories including those equipped with electronic cell counters. The following review information should help in proceeding through the cell counting protocol. Cell populations are usually expressed as the number of cells/ml or as the number of cells/culture.

When viewed with a compound microscope with a 10× ocular and a 10× objective (total magnification 100×), one large square of the haemocytometer of 1 × 1 mm will fill the field. When using a Neubauer haemocytometer, each of the four large corner squares and the large center square are counted. When the cell count is low, 10 cells/square, all nine squares should be counted. Each large square measures 1 × 1 mm and is 0.1 mm deep. Hence, each large square has volume of 0.1 mm³ or 0.0001 ml (10–4 ml). The final calculation takes into account:

1. How you wish to express your count (cells/ml or cells/flask).
2. The dilution (amount of saline, dye or media in ml which your cells have been suspended).
3. The number of squares counted. Hence, the number of cells/ml can be calculated as:
 - Total Number of Cells Counted
 - Total Number of Squares Counted
 - Trypan blue stains dead cells and therefore allows the calculation of live cells only.

Proceed to make a 1:2 split of the culture. In order to determine the number of cells which were harvested from the flask, the culture must be centrifuged, the supernatant decanted and the pellet resuspended in 1 ml MEM. Aseptically remove 0.1 ml of cell suspension and mix while adding to a dilution tube containing 0.8 ml BSS and 0.1 ml

trypan blue (0.4%). Sterility need not be maintained from this point forward. After 3–4 minutes, remove a few drops of the cell suspension with a Pasteur pipette and load both haemocytometer chambers after putting the coverslip in place. Count the total number of cells and the number of trypan blue stained (dead) cells in each of the four corner squares and the central square. Proceed to count as follows:

1. For total cell number in dilution tube (1 ml), it is calculated as follows: Total Cell Count 3×10^4 divided by 5 (number of squares counted)
2. For non-viable cell number in dilution tube (1 ml), it is calculated as follows: Total Stained Cell Number 3×10^4 divided by 5
3. For total number of cells harvested from the original flask, it is calculated as follows: Total Cell Count 3×10 (dilution factor*) $\times 10^4$ divided by 5
*A dilution or multiplication factor of 10 is used here because the aliquot removed for counting (0.1 ml) is one tenth of the total cell suspension in the centrifuge tube.
4. For number of cells/new culture after inoculation of the flask with 0.4 ml of cell suspension and addition of 5.6 ml MEM, it is calculated as follows: Total Cell Count 3×4 times 10^4 divided by 5

Cell Freezing

At BioConcept Ltd., we store our cell lines in cryotubes designated “Seed Stock” and they are frozen as soon as possible after packaging. The freezing of cells is used to preserve seed stocks of any given culture, to guard against “genetic wandering” and contamination, and for long term storage of a culture not in regular use.

BioConcept Ltd. recommends that after obtaining a fresh stock of cells from primary sources, a proportion of the culture material be preserved and frozen as early as possible. Ideally, this should be done within 1 or 2 passages after obtaining the cell culture. A “seed” stock of culture material may be withdrawn at intervals, thereby ensuring a constant, identical source of cells for many years to come. From the seed stock, further aliquots are taken, frozen and designated as “working stock.” This “working stock” has sufficient ampoules for approximately one year. When the “working stock” has been depleted, another ampoule of seed stock is thawed and grown up to provide a new supply of working stock.

Using this procedure, almost unlimited supplies of genetically similar material can be ensured. We do not recommend handling cells for more than 10 passages or 10 weeks in the laboratory. This ensures that changes will not occur in the cell line. It is essential that fresh back-up stocks always be available in deep-freeze. When freezing cells, the following recommendations ensures high cell viability:

1. Check that cells are rapidly growing and in a phase of exponential growth before freezing. If working with finite, fibroblast cultures, they should NOT have been “confluent” for more than 24 hours. If working with a continuous cell line, harvest at 70–80% confluency. Suspension cells should be spun and harvested while in exponential growth.
2. Trypsinize the monolayer as quickly as possible, using cold trypsin to minimize trypsin carry over. Use only minimal amounts of trypsin, ensuring that most is removed from the monolayer. This may be achieved by simply pouring off the trypsin or by centrifuging the cells and resuspending them in fresh medium.
3. Freeze the cells at a concentration between 2 and 5×10^6 cells/ml. For finite cultures, it is recommended that the entire contents of a flask be harvested, spun and the cell pellet resuspended in sufficient medium to produce one ampoule of

material (i.e. enough to generate one flask worth of cells at passage number 15). If two ampoules are produced from one flask, then this must be accounted for in the passage number, which in this case, a 1:2 split has been accomplished, so the passage number would be 16.

4. For established cultures, the yield is often large enough for several ampoules to be produced from one parent flask. In this case, the passage number should be increased by 1 to indicate that a passage or the equivalent of a subcultivation has occurred.
5. Keep the monolayer suspension COOL to minimize damage from the cryoprotective reagent. Since suspension cells do not respond well to low temperatures, they should NOT be cooled. Place the ampoule in a controlled rate freezer that is set to cool at a rate of 1 °C per minute. Allow the cells to freeze for approximately four hours before transferring them to a permanent position in the nitrogen freezer.
6. Frozen cells may be kept for short periods (4–10 weeks) at –20 °C, but long term storage should always be in the vapor phase of liquid nitrogen or an equivalent temperature.

Frozen Culture Recovery

Thaw the frozen ampoules by gently swirling the vial in the 37 °C water bath until there is just a small bit of ice left in the vial. Transfer the vial into a laminar flow hood, wipe the outside of the vial with 70% ethanol or isopropanol to decontaminate it prior to proceeding with the thawing procedures. Remove the cells with a narrow pipette or syringe and transfer them drop wise over 1–2 minutes to a centrifuge tube or a culture flask filled up with pre-warmed complete medium appropriate for your cell line. A 1:10 dilution should be made. Centrifuge the cell suspension at approximately 200 × g for 5–10 minutes. The actual centrifugation speed and duration varies depending on the cell type. Gently resuspend the cell pellet in fresh growth medium and transfer them into the appropriate cell culture vessel. If the cryoprotective reagent is NOT removed by centrifugation it is recommended to feed the cell culture after 24 h incubation with the growth medium.

Subculturing Protocol Established, Anchorage-Dependent Cell Lines (Long Method)

1. Aseptically decant growth medium from the flask and replace with EBSS without calcium and magnesium (Cat. No. 3-01F29-I) as follows:
 - a. 25 cm² flask approximately 5 ml
 - b. 75 cm² flask approximately 10 ml
 - c. 120 cm² flask approximately 25 ml
2. Carefully wash the monolayer with a salt solution to remove any excess serum.
3. Aseptically decant the salt solution and replace with an equal amount of 0.05% trypsin/0.02% EDTA solution (Cat. No: 5-51F00-H).
4. Pass the trypsin/EDTA solution over the monolayer several times by gently racking the flasks and decant. DO NOT allow the trypsin/EDTA to be in contact with the monolayer longer than 30 seconds.
5. Lay the flask flat and incubate at room temperature until the cells detach. Flasks which have been trypsinized should be inspected regularly to prevent the cells from remaining in the trypsin/EDTA longer than is needed.
6. Wash the cells off the base of the flask with 10 ml of the required growth medium, taking special care with cells which may still be adhering to the sides and shoulders.
7. Aspirate the cell suspension carefully to break down any cell aggregates. Avoid excessive frothing.
8. Proceed as per experimental protocol or split cells as required for stock.
9. At all times, be aware of the possibility of cross-contamination. NEVER mix caps or reuse a pipette.

References

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4. Barile, M. F., Hopps, H. E. and Grabowski, M. (1978) Incidence and source of mycoplasma contamination: a brief review, In: "Mycoplasma Infection of Cell Cultures" (McGarrity, G.J., Murphy, D.G., and Nichols, W.W., Eds.), pp. 35–46. Plenum Press, New York.
5. McGarrity, G. J. (1976) Spread and control of mycoplasma infection of cell cultures. *In Vitro* 12:643–648; 1976

Standard Packaging and Flexibility

BioConcept Ltd. has a large variety of containers available to our customers. When packaging liquid media we fill 650 ml bottles for the standard 500 ml order and for 100 ml orders we use the smaller 125 ml bottles. The Flexboy® is used for orders between 1 L and 50 L and the Flexel® is filled for those between 100 L and 500 L.

We handle all kinds of containers when packaging powder media, this includes securibox's for sizes up to approximately 1 kg. Plastic containers are used for orders up to 25 kg and for greater quantities plastic drums are adopted. The price of packaging is included with the product, so there are normally no additional costs.

At BioConcept Ltd. we are proud of our ability to listen to our customers and offer flexibility in meeting their needs. Therefore, customized packaging is also available. If you have specific container requirements please specify when placing your order.

1



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1 Sterile Bags

Sterile Bags are a single-use alternative to glass, metal and plastic containers. They are an effective means to transport media, are shatter-proof and are easy to install for our customers. Furthermore, the bags have been sterilized using gamma radiation to ensure the media does not become contaminated. Flexboys are fully compliant with ISO11137, the highest sterility assurance level.

2 Transfer Sets (also customized)

Delivery of medium from media-bags to e.g. bioreactors requires various tubings and transfer-sets. BioConcept Ltd. offers solutions according to customers specifications.

3 Plastic Drums

BioConcept Ltd. uses plastic drums made by the respected MIPI® association. MIPI is an exclusive group of packaging companies and offer localised support to businesses. The material is made from a high quality plastic and extensive tests are carried out to ensure the containers are secure. The quality standards are also in accordance to the UN's Recommendations for the Transport of Dangerous Goods, to ensure security. The drums are reusable and also recyclable, to reduce waste and an environmental impact. At BioConcept Ltd. large quantities of powders are shipped using such containers.

4 Flasks

BioConcept Ltd.'s flasks are manufactured by the Thermo Scientific company. The material used to make the flasks are strong, leak proof and ideal for cell culture media. The shape of the flasks also make for easy storage in bulk and as individual flasks. A lot of BioConcept Ltd.'s smaller volume orders of cell culture media are shipped in such containers. The flasks are sterilized before entering the clean room and are recyclable.

5 SecuriBoxes™

For smaller orders of our powder medium, or on the customer's request, the product is packed into Securiboxes. The containers are strong and are the suitable dimension for easy storage and use. The containers are created by the Joma company in Austria in an ISO 7 production facility. The boxes are rigorously tested according to pharmaceutical industry guidelines for ISO 15378:2207.

6 Transport Container

The large metal containers seen in this photograph hold 500 liter media bags. The metal container protect the bags from any potential puncturing or damage during storage and delivery. Once the bags are filled in the sterile room, they are quickly placed in the containers and taken to the storage facilities, where they are kept at the appropriate temperature needed for the media. The containers are stored for fourteen to twenty eight days to allow time for quality checks.

If you have any questions then please contact us by e-mail: info@bioconcept.ch

Protocols

Reconstitution of MAM-PF[®]7e Powder (Animal Component Free) Results in MAM-PF77 liquid

Required Supplements

1. L-Gln (200 mM)
2. Lipid Mix (1 000 ×) sterile, (Cat. No: 5-77F02)
3. Supplement 1 for 10-02P74 (Cat. No: 5-09Z08)
4. Supplement 2 for 10-02P74 (Cat. No: 5-09Z12)
5. MAM-PF77 Suppl. 1 (Cat. No: 5-03Z78)
6. MAM-PF77 Suppl. 2 (Cat. No: 5-03Z79)
7. MAM-PF77 Suppl. 3 (Cat. No: 5-03Z80)
8. NaHCO₃ (1.945 g/L)

Reconstitution of MAM-PF[®]7e Powder (Cat.No. 10-02P74) Results in 1 L liquid (10-02S80)

1. Dissolve 23.80 g powder (Cat. No: 10-02P74 in 900 ml bidistilled water or equivalently purified H₂O)
2. Add L-Glutamine (200 mM) to required final concentration (recommended 0.6–8 mM)
3. Add 1 ml Lipid Mixture (1 000 ×), (Cat. No: 5-77F02)
4. Add 1.85 ml Supplement 1 for 10-02P74 (Cat. No: 5-09Z08)
5. Add 20 ml Supplement 2 for 10-02P74 (Cat. No: 5-09Z12)
6. Add 2 ml MAM-PF77 Supplement 1 (Cat. No: 5-03Z78)
7. Add 1ml MAM-PF77 Supplement 2 (Cat. No: 5-03Z79)
8. Add 1 ml MAM-PF77 Supplement 3 (Cat. No: 5-03Z80)
9. Add 1.945 g/L NaHCO₂
10. Adjust pH to 7.10–7.20
11. Add bidistilled water (or equivalently purified H₂O) to final volume (1 L medium)
12. Filtration: 0.2 µm filter

Express Media for Hybridoma Cells

Product Handling

The optimal culture conditions are 37 °C with 5 % CO₂. When cultured in flasks, the lid should be slightly opened for gas exchange.

Adaptation of cells to serum-free conditions

In most cases is a direct cultivation of hybridoma cells in ISF-1 possible. Nonetheless, the following protocol for a stepwise adaptation has been proven successful. The successful adaption also depends on the character of the used hybridoma cell line. Therefore, it is highly recommended that a control culture is continued to be kept in its original medium until the adaptation to ISF-1 has been successful. The cell culture should be in its logarithmic growth phase with a minimum rate of living cells of 90 %.

Direct adaptation

1. Subculture of cells from serum containing media into 37 °C pre-warmed ISF-1. Cell density should correspond to the one used in the original culture.
2. Incubation of cells at 37 °C with 5 % CO₂.
3. Passaging of cells under strict control of its growth curve and viability for a minimum of 4–8 passages.
4. If growth and viability is significantly reduced during the 4–8 passages, the stepwise adaptation protocol should be used.

Stepwise adaptation

1. Seeding of cells with double the density compared to its normal inoculum into a 75:25 (v/v) mixture of serum containing to serum-free medium.
2. Subculture of cells when density reaches 10⁶ viable cells/ml into a mixture of 50:50 (v/v) serum containing to serum-free medium.
3. Subculture of cells when density reaches 10⁶ viable cells/ml into a mixture of 25:75 (v/v) serum containing to serum-free medium.
4. Subculture of cells when density reaches 10⁶ viable cells/ml into ISF-1.

SERUM FREE CULTURE in SF-4 Baculo Express Medium

SF-4 Baculo express medium is a proprietary formulation which has successfully been used to grow various *Spodoptera frugiperda* (SF9, SF21), BTI-TN-5B1-4 (High Five™) and *Drosophila melanogaster* (D.Mel-2) cells.

1. Guidelines for adaptation to SF-4

Special regimens are required to adapt insect cells from serum-containing to serum free SF-4 media: direct and gradual replacement (weaning). It is critical that the cells are in exponential growth before medium replacement and that the minimum cell density is at least 2×10^5 cells/ml medium.

a. Direct medium replacement

1. Grow the cells to about 60% confluence (anchored cultures) or a density of 2×10^6 cells/ml (suspension cultures) in serum containing media.
2. Harvest the cells, dilute to 5×10^5 cell/ml serum-free SF-4 medium and grow in suspension at 27 °C.
3. After they have reached a concentration of $2-4 \times 10^6$ cells/ml (normally after 5–6 days) dilute with fresh SF-4 medium to 2×10^6 cells/ml and repeat this procedure. After two to three cycles the cells are adapted to serum-free growth.

b. Gradual medium replacement

1. Seed cells in a tissue culture flask or a Petri dish at a concentration of 5×10^5 cells/25 cm² surface in 3.6 ml serum containing medium. After 1 day add $1/10$ volume of SF-4 medium and grow the cells to 60% confluence.
2. Subculture in 3 ml serum containing medium and SF-4 medium (9:1 v/v) and add after one day volume SF-4 medium.
3. Subculture in 3 ml serum containing and SF-4 medium (3:1 v/v) and add after one day. volume SF-4 medium.

2. Guidelines for specific growth conditions

a. Monolayer culture

Using a pipette aspirate medium and floating cells from a confluent monolayer, discard and again add about 4 ml of fresh complete medium to a 25 cm² flask. Resuspend cells by pipetting the medium across the monolayer. Observe cell monolayer to ensure complete cell detachment from the surface of the flask. Perform viable cell count on harvested cells (e.g. using trypan blue). Inoculate cells at around 1.2×10^6 cells/25 cm² flask. Return cultures to incubator (27 °C ± 0.5 °C). On day three post-planting aspirate the spent medium from one side of the monolayer and re-feed the culture with fresh medium gently added to the side of the flask.

b. Spinner

Recalibrate the spinner flasks using a graduated cylinder or volumetric flasks as a reference. Calibration is performed with the impeller apparatus removed from the vessel. Impeller mechanisms must rotate freely, do not allow contact with vessel walls or base. Avoid physical stress as most invertebrate cells are sensitive to physical shearing. Adjust the spinner mechanism so that paddles clear sides and bottom of the vessel (adjust prior to autoclaving). Four to six confluent 75 cm² monolayer flasks are needed to initiate a 100 ml culture (4–5 flasks for the spinner culture and one as a backup). Dislodge cells from the base of the flasks as described in a. (monolayer culture). Pool the cell suspension and perform a viable cell count. Dilute the cell suspension to approximately 3×10^5 viable cells/ml in complete medium. For culture volumes of 75–100 ml, use a 100 ml spinner vessel. For volumes of 150–200 ml, use a 250 ml vessel. Stock cultures should be maintained in a 150 ml culture in a 250 ml spinner vessel. The top of the paddles

will be slightly above the medium, which provides additional aeration to the cultures. Atmospheric gas equilibration is accomplished by loosening the side arm caps on the vessels (about $1/4$ turn). Incubate spinner vessels at 27 °C ± 0.5 °C at a constant stirring rate of 75 rpm. Re-seed spinner cultures to approximately 3×10^5 cells per ml twice weekly in well-cleaned, sterile vessels. Once every two weeks spinner cultures may be gently centrifuged at $100 \times g$ for 5 minutes and resuspended in fresh medium to reduce accumulation of cell debris and toxic metabolic by-products.

c. Shaker culture

The orbital shaker apparatus should have a capacity of up to 135 rpm. As standard flask use the 250 ml disposable sterile Erlenmeyer flask. The orbital shaker/flask assembly should be maintained in a 27 °C ± 0.5 °C non-humidified, non-gas regulated environment.

Aeration is accomplished by loosening the cap approximately $1/4$ turn (within the intermediate closure position). In this condition, there is no oxygen limitation to the cells and they therefore proliferate with maximal rates.

Inoculate a 250 ml Erlenmeyer flask with 100 ml of complete medium containing 3×10^5 viable cells per ml. Set the orbital shaker 125–135 rpm. Subculture to approximately 3×10^5 cells/ml twice weekly. Every three weeks, cultures may be gently centrifuged at $100 \times g$ for 5 minutes and pellets resuspended in fresh medium to reduce accumulation of cell debris and toxic metabolic by-products. As cultures may be passage number dependent, fresh cultures should be established from frozen seed stocks every three months.

Guidelines for virus and recombinant protein production

Anchored cells

1. Seed the cells in a concentration of 2×10^6 cells/25 cm² flask. Cell normally attach within 15 min but firmly after 1 h.
2. After 1 day remove the medium and incubate the cells for 1 h, with gentle rocking, with 0.5 ml of virus suspension containing $4-8 \times 10^7$ tissue culture infective dose 50% (TCID₅₀) units per cell, giving a multiplicity of infection of about 10–20 TCID₅₀ units per cell.
3. Wash the cells after removal of the medium and add 4 ml of fresh medium.
4. Isolate infectious virus, polyhedra or recombinant proteins from 48 h post infection onwards. After 72 h post infection, cell start to lyse.

Suspension cultures

1. Centrifuge cells of a suspension culture in logarithmic growth (500 g for 5 min).
2. Resuspend the cells to a density of 10^7 cells/ml in virus containing medium. This medium should contain $1-2 \times 10^8$ TCID₅₀ units of virus to give a final multiplicity of infection of 10–20 TCID₅₀ units per cell.
3. Isolate infectious virus, polyhedra or recombinant proteins from 48 h post infection onwards. After 72 h post infection, cell start to lyse.

Freezing

Prepare desired quantity of cells in either spinner or shaker culture, harvesting in mid log phase of growth at a viability of >90%. Determine the viable cell count and calculate the required volume of cryopreservation medium required to yield a final cell density of 0.5 to 1.0×10^7 cells/ml. Prepare the required volume of cryopreservation medium (7.5% DMSO and 10% BSA or FCS) in SF-4 Baculo Express medium. Hold

medium at +4 °C. Pellet cells from culture medium at 100 × g for 6 minutes. Re-suspend pellet in the determined volume of +4 °C cryopreservation medium. Incubate cell suspension at +4 °C for 30 minutes (until well chilled). Dispense aliquots of this suspension to cryovials. Frozen cells are stable indefinitely under liquid nitrogen.

Recovery

Recover cultures from frozen storage by rapid thawing a vial of cells in a 37 °C water bath. Transfer the entire contents of the vial into a 250 ml shaker flask containing 100 ml complete growth medium and incubate culture as described in shaker culture. Maintain culture between 3×10^5 and 1×10^6 cells/ml for the first two subcultures after recovery, and then returning to the normal maintenance schedule.

Human Mesenchymal Stem Cell (hMSC) chondrogenesis induction medium

Culture protocol

- Estimate the total number of pellet cultures needed for your experiment. Beware: 2.5×10^5 cells are required to form one chondrocyte pellet.
- After harvesting your pre-cultured hMSCs, transfer 2.5×10^5 cell for each pellet into a separate 15 ml polypropylene tube.
- Centrifuge the cells at 150 × g for 5 min and discard the supernatant. Resuspend the cells in 500 µl of chondrogenesis control (without TGF-β3) or induction media (with TGF-β3).
- Centrifuge the cells again at 150 × g for 5 min and DO NOT aspirate the supernatant, and DO NOT resuspend the pellet!
- Loosen the cap of the tubes one half turn to allow gas exchange and incubate the tubes at 37 °C, 5 % CO₂ for 48 h.
- Meanwhile, the cells should have formed a spherical aggregate detached from the tube wall.
- Replace every 2–3 days 90 % of the supernatant with 450 µl of the corresponding medium.
- Having replaced the media, gently flick the bottom of each tube to ensure the pellet is free floating, and do not forget to loosen the cap of the tube again before returning it to the incubator.
- Chondrogenic pellets and also the control pellets should be harvested after 28 days in culture.

Human Mesenchymal Stem Cell (hMSC) osteogenesis induction medium

Culture protocol

- After harvesting your pre-cultured hMSCs, plate 5000 cells/cm² in a 6 well-plate (1 well ~ 10 cm² = 5×10^4 cells/well).
- Feed the cells every 2–3 days with hMSC proliferation media until the culture reaches 100 % confluence (approx. 5–7 days).
- Having 100 % confluent hMSC cultures, change the media all 2–3 days with osteogenesis induction media and incubate the cells always at 37 °C, 5 % CO₂.
- Possible negative controls will be fed with osteogenesis induction media without the osteogenesis induction factor (that means basal media only supplemented with HEPES, FCS and L-Glutamine).
- After at least 28 days of culturing osteogenic structures can be detected e.g. with Von Kossa or Alkaline phosphatase staining.

Human Mesenchymal Stem Cell (hMSC) adipogenesis induction medium

Culture protocol

- Prepare the following media:
 - Adipogenesis maintenance media (AMM): Add one vial of Insulin to 50 ml of basal media (already supplemented with FCS, HEPES and L-glutamine).
 - Adipogenesis induction medium (AIM): Add one vial of Dexamethasone, one vial of Indomethacine and one vial of 3-isobutyl-1-methyl-xanthine to 50 ml of basal medium (already supplemented with FCS, HEPES and L-glutamine).
- After harvesting your pre-cultured hMSCs, plate 5000 cells/cm² in a 6 well-plate (1 well ~ 10 cm² = 5×10^4 cells/well).
- Feed the cells every 2–3 days with hMSC proliferation media until the culture reaches 100 % confluence (approx. 5–7 days).
- Having 100 % confluent hMSC cultures, 3 cycles of inductions and maintenance follow:
 - 1st cycle: culture in AIM for 3 days followed by culture in AMM for 2 days.
 - 2nd cycle: culture in AIM for 3 days followed by culture in AMM for 2 days.
 - 3rd cycle: culture in AIM for 3 days followed by culture in AMM for 2 days.
- Change media every 3rd or 2nd day according to the cycles shown above with fresh AIM or AMM and incubate the cells at 37 °C, 5 % CO₂.
- Possible negative controls will be fed with AMM.
- After 15 days of culturing the cells should show lipid vacuoles, which can be detected with Oil Red O staining.



Index

Alphabetical Product Index

Numerical Index

Nudibranch [*Nudibranchia*]

Nudibranchs are a large group of mollusks which are known for their often extraordinarily vibrant colours and bizarre appearances. There are over 3000 species of Nudibranchs, whose name comes from the latin word nudus, meaning naked, referring to the fact it sheds its shell after the larva stage. It is logical to think that their lack of shell would make them an easy target, however they are a relatively successful group. This is partially due to their bright colours warning off hungry predators, and partially because a number of types produce chemicals which make them taste terrible.



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