

A close-up photograph of a clownfish (Amphiprioninae) partially hidden within the tentacles of a sea anemone. The anemone's tentacles are thick and cylindrical, with a color gradient from light yellow to pale blue. The clownfish has a white stripe running vertically down its side and is looking towards the camera with its mouth slightly open.

SPECIAL MEDIA GUIDE

Hybridoma Express Media

Insect Cell Culture Media

SF-4 Baculo Express Medium

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

Stem Cell Media

DISTRIBUITO DA





Who we are

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.

The strength and focus of BioConcept Ltd. lies in manufacturing customized cell culture media as well as defined media for recombinant protein production. Beside 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. Our water preparation facilities are specifically engineered to efficiently generate the highest standard Water For Injection (WFI) available.



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 (Animal Component Free) 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 5000 L (liquid) and 2 kg up to 800 kg (powder)
- Variable packaging sizes (1 ml up to 1000 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.

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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).

Available Hybridoma Growth Media

| 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 |

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

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 of the main catalogue |
| 1-43F00-I | Schneider's <i>Drosophila</i> Medium w/o L-Gln | 500 ml | | × | See page 59 of the main catalogue |
| 1-43F02-I | Schneider's <i>Drosophila</i> Medium with L-Gln | 500 ml | | × | See page 59 of the main catalogue |
| 1-34F00-I | Mitsuhashi and Marmorosh | 500 ml | Contains Lactalbumin- hydrolsate and yeast extract | | See page 58 of the main catalogue |

Other modifications are available upon request 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.

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

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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.

Available BSK-H Media

| Cat. No | Description | Size |
|-----------|----------------------------------|--------|
| 1-10S02-H | BSK-H Medium with L-Glutamine | 100 ml |
| 1-10S02-I | BSK-H Medium with L-Glutamine | 500 ml |
| 1-10S03-I | BSK-H Medium without L-Glutamine | 500 ml |

Stem Cell Media and Supplements

Human Mesenchymal Stem Cell (hMSC) proliferation medium

Media for culturing hMSC delivered as a basal medium with supplements.

| 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.

| 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 |

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.

| 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 |

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.

| 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 |

Cancer Stem Cell Medium

Media for culturing human cancer stem cells delivered as a basal medium with supplements.

| 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 |

All stem cell media formulations are 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.

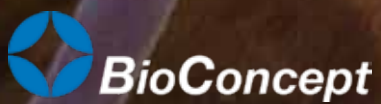
BIT 9500 Serum Substitute

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 °C) or overnight at 2–8 °C. Swirl the bottle to mix its content. Store at 2–8 °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.

| Cat. No | Description | Size |
|-----------|---------------------------|--------|
| 5-18S02-H | BIT 9500 Serum Substitute | 100 ml |



Paradiesrain 14
Postfach 427
CH-4123 Allschwil 1
Switzerland
Tel. +41 (0)61 486 80 80
Fax +41 (0)61 486 80 00
info@bioconcept
www.bioconcept.ch

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MAM-PF[®]

CHO PRODUCTION MEDIA

Benefits at a glance:

- ACF (Animal Component Free)
- Chemically defined
- High cell densities
- Pharmaceutical application
- High product yield

History

Forty years of experience serving the Swiss biological community.

2015 the brand new liquid media production plant was opened.

1993 BioConcept added the Amimed® brand to its portfolio.

BioConcept has developed a strong international presence with its Amimed® brand.

BioConcept has a positive reputation because the company is constantly updating its practices in order to keep up with the advancing field of cell biology.

Flexibility

BioConcept is a privately held company.

BioConcept listens to the customers' needs and does its utmost to meet them.

Powder and liquid media plants.

Batch sizes from 5 up to 5,000 Litres. 1 kg powder up to 800 kg.

Production with WFI (Water For Injection).

Media can be delivered within a few weeks if needed.

Facilities

In 2015 BioConcept opened its brand new liquid media plant. The plant was designed to uphold a maximum degree of sterility through its state of the art air processing system and advanced machinery.

More information:

www.bioconcept.ch/de/Downloads

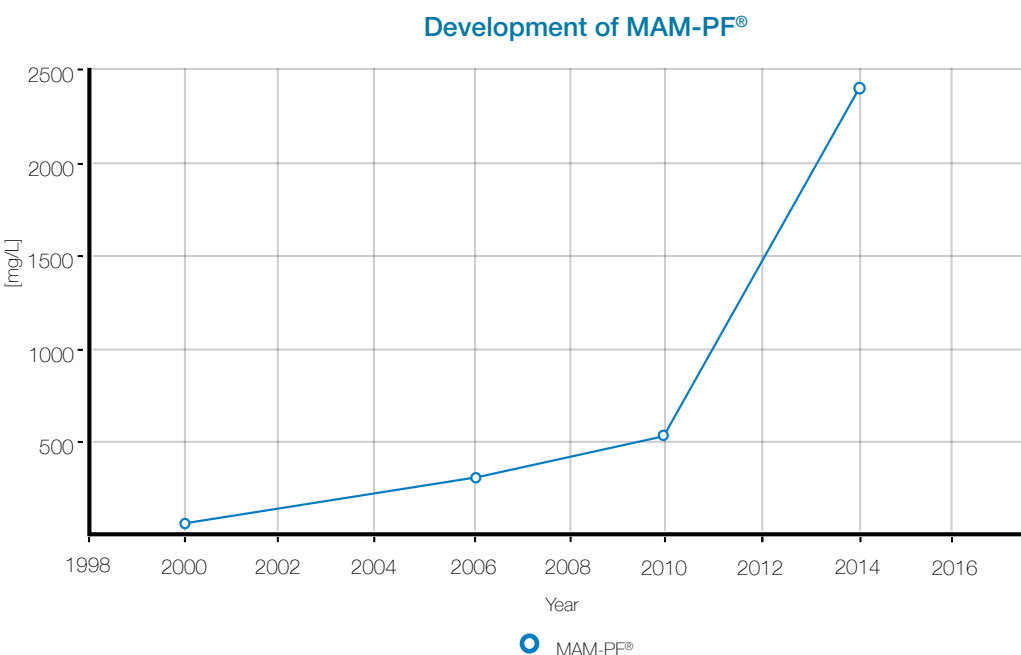




Highlights

of the MAM-PF® series

- Animal Component Free**
MAM-PF® media do not contain proteins or undefined hydrolysates.
- Liquid batch capacity**
Batch sizes ranging from 5-5000 litre, water for injection (WFI) is the highest quality available.
- Chemically defined**
BioConcept holds TSE certificates for each component to ensure EMA/410/01 conformity.
- Powder batch capacity**
Batch sizes ranging from 1 - 800 kg, our milling process results in particle sizes of around 20 µm (d50). This leads to a quick dissolution of the powder medium.
- Easy adaptation**
In many cases it is possible to switch directly from your current medium to MAM-PF®.
- Feed mixes**
Various feed mixes are available for high density cell culture and high productivity.
- High cell density combined with high product yield**
 - Antibody production of up to 5.5 g/L.
 - EPO of up to 2.5 g/L (see graph below).
 - Cell density up to 3.7×10^7 cells/ml.
- Glycosylation**
Best glycosylation pattern observed.



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.

MAM-PF® series

MAM-PF® (Mammalian Artificial Medium - Protein Free) media are Animal Component Free (ACF) and in accordance with the strict quality guidelines EMA/410/01. MAM-PF® is a production media. It is protein-free and protein hydrolysates free, chemically defined and for high cell density cultivation of a variety of cell lines such as CHO (Chinese Hamster Ovary) cells or BHK (Baby Hamster Kidney) cells and the high level expression of recombinant proteins. BioConcept holds a certificate for every single component used in the MAM-PF® media series to guarantee an untainted and exceptional final product.

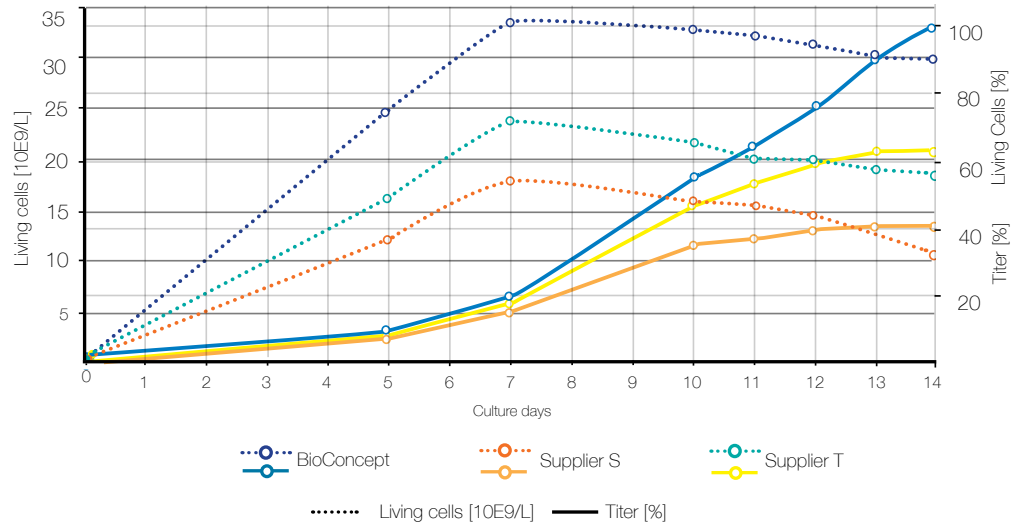
Performance

Cell Density

CHOSI cells cultured in MAM-PF77[®] have shown the fastest growth and a ~100% and ~40% higher cell density compared to media by Suppliers S and T. This corresponds with the final product titers at the end of the fed-batch, respectively (All cultures were fed with FMS3 in the same feed regime).

The higher viability in the stationary phase shows that the glycosylation in MAM-PF77[®] was superior.

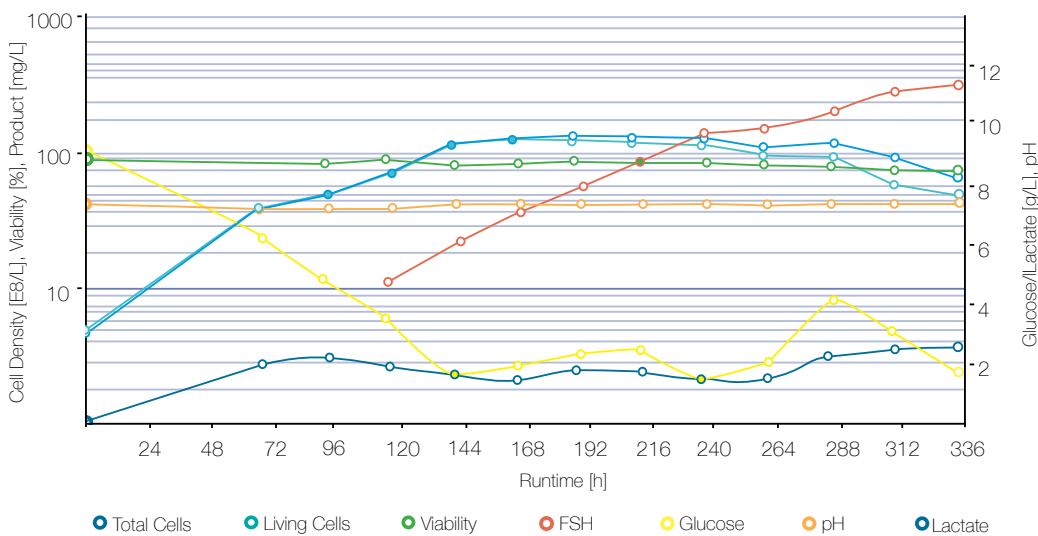
MAM-PF[®] and Other Suppliers



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



FSH

A cell density of over 100 mio. cells per liter can be reached through mixing the CHO feed mix FMS3 to MAM-PF77[®]. It is possible to achieve a titer of over 350 mg/L of the highly glycosylated follicle-stimulating hormone (FSH) within 14 days in a stirring bioreactor tank, making it a very high quality product. As determined during the purification process, 45% of the product showed an isoform-pattern, low aggregates, and low oxidized forms. MAM-PF77[®] can be used to produce quality FSH that fulfills its Ph.Eur. requirements.

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

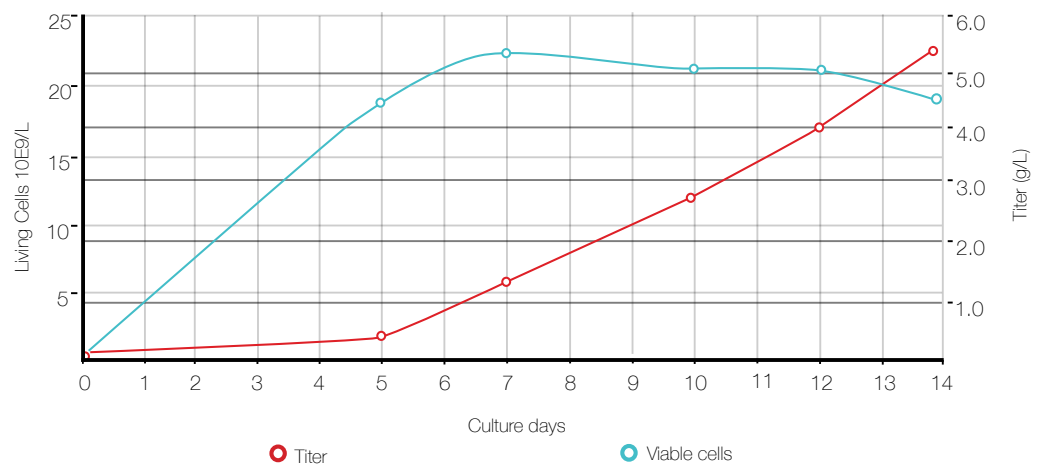
Application Data Antibody



Ipilimumab

The continuous innovation and development of the MAM-PF[®] media series has led to the brand new MAM-PF77[®] cell culture medium and CHO Feed Mixes FMS3 and FMU. MAM-PF[®] media now increase productivity of Ipilimumab (monoclonal antibody) by up to 5g/L. The new expression system is also viable in fed-batch and perfusion systems.

Ipilimumab produced with MAM-PF[®] and FSU



High yields of mAbs, e.g. > 5g/L Ipilimumab can be reached in a 14-day fed-batch system using MAM-PF77[®] plus the novel CHO feed mix FMU.



Selection of pre-developed biosimilars produced with MAM-PF® media series.

| API (Indication) | Available (EUGENEX Biotechnologies) | Brand Name (Originator) | Global Sales 2012 Estimates [Mio \$] |
|--|-------------------------------------|---|--------------------------------------|
| EPO , Epoetin alpha (Anemia) | Cell-Line & USP & DSP | Epogen (Amgen) Eprex (Johnson) | ~5.000 |
| DPO , Darbepoetin alpha (Anemia) | Cell-Line & USP & DSP | Aranesp (Amgen) | ~2.500 |
| INFb , Interferon beta 1a (MS) | Cell-Line & USP & DSP | Avonex (Biogen) Rebif (Serono) | ~1.200 |
| FSH , follicle stimulating hormon (Infertility); also hCG & LH | Cell-Line & USP & DSP | Gonal-F (Serono) Puregon (Organon) | ~500 ~300 |
| Etanercept , TNFa receptor IgG (chronical arthritis, psoriasis) | Cell Line USP & DSP | Enbrel (Amgen, Pfizer, Takeda) | ~8.400 |
| Adalimumab , TNFa Mab (rheumatoid arthritis, Crohn's) | Cell Line USP & DSP | Humira (Abbott) | ~9.300 |
| Rituximab , CD20 Mab (rheumatoid arthritis, lymphoma) | Cell Line USP & DSP | Rituxan (Roche) | ~6.900 |
| Trastuzumab , HER2 Mab (mammarcarcinom) | Cell Line USP & DSP | Herceptin (Roche) | ~6.100 |
| Bevacizumab , VEGF Mab (colorectal cancer) | Cell Line USP & DSP | Avastin (Roche) | ~6.300 |
| Cetuximab , EGF receptor Mab (colorectal cancer) | Cell Line USP | Erbix (BMS, Imclone) | ~1.000 |
| Omalizumab , IgE Mab (persistent allergic asthma) | Cell Line USP | Xolair (Genentech/Novartis) | ~1.000 |
| Denosumab , RANKL IgG (osteoporosis, colorectal cancer) | Cell Line USP | Prolia (Amgen) | ~500 |
| Eculizumab , Complement C5 Mab (hemoglobinuria (PNH)) | Cell Line USP | Soliris (Alexion) | ~1.100 |
| Ipilimumab , CTLA-4 IgG1 (metastatic melanoma) | Cell Line USP | Yervoy (BMS) | ~1.000 |
| Tocilizumab , IL-6R IgG1 (Castelman, rheumatoid arthritis) | Cell Line | Actemra (Roche, Chugai) | ~1.000 |
| Abatacept , CTLA-4-IgG1 fusion (rheumatoid arthritis) | Cell Line | Orencia (BMS) | ~1.000 |
| Pertuzumab , Her2 dimer inhibitor (metastatic breast cancer) | Cell Line USP | Omnitarg (Roche) | ~1.000 |
| Panitumumab , EGF-R Mab (colorectal cancer) | Cell Line | Vectibix (Genentech/Novartis) | ~400 |
| Ofatumumab , CD20 IgG1 2 nd Gen. (leukemia and others) | Cell Line | Arzerra (Genmab/GSK) | ~100 |

Biosimilars and MAM-PF®

Biosimilars are highly diverse and complex. The medicines are a large group that include growth factors, cytokines, hormones, monoclonal antibodies (mAb) and, potentially, vaccines (Huzair and Kale, 2015). Due to their complexity and post-translational modifications (e.g. glycosylation of mAbs), many biosimilars are produced using the CHO (Chinese Hamster Ovary) expression system. Finding a medium that meets the strict regulations set for biosimilar production and creates a highly superior product can be challenging. Nevertheless, you must look no further: At BioConcept we offer high quality products that are both fully chemically defined and animal component free. This is the groundbreaking MAM-PF® media series. In the adjoining table you will find a selection of successfully produced biosimilars that are cultured with MAM-PF® media in the designed expression CHO host cell line (propriety of EUGENEX Biotechnologies).

Selection of references for the MAM-PF media series:

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BioConcept is serving you:

Swiss Cell Culture Media

CHO cell culture media (ACF)
Insect cell culture media
Hybridoma cellculture media
Classical cell culture media
Sterile salt solutions
And more

Discover more on:
www.bioconcept.ch

Your local distributor:

DISTRIBUITO DA



BioConcept Ltd.
Paradiesrain 14
Postfach 427
CH-4123 Allschwil/BL
Switzerland

Tel: +41 (0)61 486 80 80
Fax: +41 (0)61 486 80 00
Web: www.bioconcept.ch
E-Mail: info@bioconcept.ch

Version Nr: CHO20160421

| | |
|---|--|
| <p>HYGM-6</p> <p>Hybridoma growth medium SERUM free</p> <p>Cat. No: 9-00F55-I Cat. No: 9-00F57-I (without Phenolred)</p> <p>HYGM-6 Medium is a serum free medium. Insulin rec. for therapeutic use is the only protein in that medium, no other proteins or undefined hydrolysates.</p> <p>Size: 500 ml</p> | <p>HYGM-7</p> <p>Hybridoma growth medium PROTEIN free</p> <p>Cat. No: 9-00F56-I Cat. No: 9-00F58-I (without Phenolred)</p> <p>HYGM-7 Medium is a totally chemical defined medium and does not contain any undefined hydrolysates or proteins</p> <p>Size: 500 ml</p> |
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HYGM-6 and HYGM-7

Serum and Protein-Free Media for Hybridoma Cell Culture and Monoclonal Antibody Production

Media are ready-to-use and already supplemented with stable Glutamin, no further supplementation required.

**Storage Conditions: 2 to 8 °C in the dark.
Alternative packaging on request**

Intended Use

Hybridoma growth media (HYGM) for hybridoma cell culture have been designed and optimised for the serum-free growth of a variety of hybridoma cell lines and production of monoclonal antibodies.

Introduction

Traditional hybridoma culture media requiring serum supplementation have in recent years been replaced by a variety of commercially available serum-free formulations. Many serum-free formulations contain proteins (e.g., insulin, transferrin, albumin) and/or protein hydrolysates and lysates.

BioConcept po box 427 CH-4123 Allschwil

Tel.: +4161-4868080 Fax: +4161 4868000

www.bioconcept.ch info@bioconcept.ch

As a result of a trend towards greater levels of media definition and the need for replacement of components of animal origin with non-animal derived materials, many serum-free media formulations are considered unacceptable for certain applications. HYGM-7 is a protein-free product for growth of hybridomas and monoclonal antibody production. HYGM-6 is a serum-free medium that supports growth and monoclonal antibody production of a variety of hybridoma cell lines.

Precautions

Addition of antibiotics should not be used as a substitute for proper sterile technique. In most instances, antibiotics are neither necessary nor advised. However, in those instances where antibiotics are desired, most general antibiotics are compatible with our Hybridoma Media including penicillin/streptomycin, gentamicin, anti-PPLO and amphotericin B.

Instructions For Use

Physical Conditions

37 °C +/- 0.5 °C in a humidified atmosphere of 5 - 8 % CO₂ in air. Caps of flasks should be loosened to permit gas exchange. Cultures may be grown in stationary suspension culture (e.g., T-flask) or in agitated suspension culture (shaker or spinner flasks). Adequate headspace should be provided to facilitate gas exchange. (e.g., for a 125 ml shaker flask, use no more than 35 ml culture volume). Shaker flasks should be rotated at 125 - 135 rpm; agitation speed in spinner flasks will depend upon the impeller design. Protect cultures from light.

Adaptation of Cells to Serum-free or Protein-free Media

A sequential adaptation protocol may be necessary if direct adaptation does not work. In both cases, the cells should be in mid-logarithmic growth phase with high (>90%) viability. Success of the adaptation method will depend upon the particular hybridoma cell line and the culture conditions employed. It is recommended that backup cultures in the original medium be maintained until success with the new medium has been achieved.

A. Direct Adaptation

1. Transfer hybridoma cells growing in serum supplemented medium to serum-free medium which has been prewarmed to 37 °C. Seeding density should be double the normal seeding density for the cell line. Incubate the cells at 37 °C in a humidified atmosphere of 5-8% CO₂ in air.
2. Monitor cell growth until viable cell density reaches 1 x 10⁶ /ml. Subculture the cells to a viable cell density of 1-2 x 10⁵ /ml in fresh serum-free medium. Subculture in this manner, monitoring cell growth and viability, for 3 to 5 passages.

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3. If the culture fails to maintain acceptable growth and viability over 3-5 passages during direct adaptation, use the sequential adaptation method.

B. Sequential Adaptation

1. Inoculate hybridoma cells at double the normal seeding density in a 75:25 (v/v) mixture of serum supplemented : serum-free medium.
2. Monitor the culture until the density reaches 1×10^6 viable cells/ml. Then subculture into a 50:50 (v/v) mixture of serum supplemented : serum-free medium.
3. Monitor the culture until the density reaches 1×10^6 viable cells/ml. Then subculture into a 25:75 (v/v) mixture of serum supplemented : serum-free medium.
4. Monitor the culture until the density reaches 1×10^6 viable cells/ml. Then subculture into 100% serum-free medium.
5. It may be necessary to subculture more than once into a given mixture of serum supplemented : serum-free medium until the cells become acclimated. It is advisable to keep a backup culture in the previous media mixture until the cells have adapted.

Cryopreservation

1. Prepare desired quantity of cells, harvesting in mid-log phase of growth with viability higher 90%.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium (50% fresh medium : 50% conditioned medium + DMSO to a final concentration of 7.5%) to give a final cell density of $0.5 - 1.0 \times 10^7$ cells/ml. Conditioned medium should be obtained from a high viability, mid-log culture of cells.
3. Prepare the required volume of cryopreservation medium and hold the medium at 4°C until use (make cryopreservation medium on day of intended use).
4. Pellet the cells from culture medium at 100 x g for 5 minutes. Resuspend the pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications.
6. Achieve cryopreservation in either an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Frozen cells are stable indefinitely under liquid nitrogen.

Recovery from Cryopreservation

1. Recover cultures from frozen storage by rapid thawing of a vial of cells in a 37 °C water bath with shaking just until the medium thaws.
2. Transfer the entire contents of the vial into the appropriately sized vessel so that the cells are seeded at 5×10^5 cells/ml of complete growth medium.
3. Incubate the culture in a humidified atmosphere of 5-8% CO₂ in air at 37+/-0.5°C. Do not centrifuge the cells as they are extremely fragile upon recovery from cryopreservation.
4. Maintain the culture between 5×10^5 and 10×10^5 viable cells/ml for the first two subcultures following recovery; thereafter, returning to the normal maintenance schedule.

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Quality Control

HYGM-media for hybridoma applications are performance tested using a hybridoma cell line. Additional standard evaluations are pH, osmolality and sterility tests according to USP or Pharma Eur.

Further information:

info@bioconcept.ch

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Custom Media Configurator - We offer you a convenient way to produce your custom media quickly and cost-efficiently. Fill in the media configurator below and we will revert back with your media development and production costs.

| | | | |
|--|--|---|--------------------------------|
| Product name | Click here to enter text. | | |
| Medium is | <input type="checkbox"/> ACF/AOF (Animal Component-Free; Animal Origin-Free) <input type="checkbox"/> non-ACF <input type="checkbox"/> Chemically Defined (CD) | | |
| Copy paste your formulation here in the box or attach it using the upload button | | <div style="border: 1px solid #ccc; padding: 10px; display: inline-block;">Upload</div> | |
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| Order/Batch Size (volume in Litres) | Click here to enter text. | | |
| Total Annual demand (in Litres) Note: Our minimum order quantity for special media is 20 liters. Special productions are subject to a 10% under/overage. | Click here to enter text. | | |
| Target price (per Litre) | Click here to enter text. | | |
| Which filtration level? | <input type="checkbox"/> 0.2um | <input type="checkbox"/> 0.1um | <input type="checkbox"/> other |
| Filling | <input type="checkbox"/> bottle | <input type="checkbox"/> bag | |
| Combinations are possible (enter in box) Bottles:100 ml,500 ml, 1000 ml 2D: 1 L, 5 L, 10 L, 20 L, 50 L 3D: 100 L, 200 L, 500 L, 1000 L | Click here to enter text. | | |
| Labelling | <input type="checkbox"/> Own label (supplied by customer) <input type="checkbox"/> Our Label | | |
| QC testing | <input type="checkbox"/> Standard (osmolarity, sterility, pH, endotoxin, cell growth, conductivity, bioburden) <input type="checkbox"/> Mycoplasma <input type="checkbox"/> Nucleases <input type="checkbox"/> Stability <input type="checkbox"/> Other Click here to enter text. | | |
| Shipping | <input type="checkbox"/> Ship using my own courier account (ex-works; EXW) <input type="checkbox"/> Ship with courier and add shipping charges to the invoice | | |
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