1. Serum-free Systems

Introduction

Basics:

Cell culture, the cultivation of cells isolated from live tissue in vitro (in the test tube), is an acknowledged and valuable tool in the biomedical research for the determination of reproducible data. Apart from that, by means of cell cultures more and more highly effective substances are produced in large-scale for medicine and research (e.g. Insulin, Growth Factors, Monoclonal Antibodies or Clotting Factors).

The Cell Culture with Serum:

Cells in vitro need nutrient solutions, so-called media which guarantee an imitation as exact as possible of the situation in vivo (in live organism). On the other hand these media – mixtures of nutrients, salts, trace elements, buffer substances, growth factors, protective substances and many other components – must be complemented for this purpose by the most natural, highly complex additives. Not long ago animal but also human serum were the means to be chosen for reasons of production technology and also because of a lack of alternatives.

Function of Serum in the Cell Culture:

- Hormone factors stimulate cell growth, proliferation and differentiation.
- Attachment factors favour or enable the attachment of the cells to the culture dish (Biomatrix).
- Transport and binding proteins take care among other things of the supply of hormones, minerals and lipids.
- Serum proteins bind toxic substances.

This serum, mostly fetal bovine serum (FCS), is problematic for several reasons.

Disadvantages of Serum in the Cell Culture:

- The composition of serum is not constant and varies with the age of the foetus, with the origin and feeding of the animals and with the time of the year.
- Serum batches have to be tested for their suitability before use.
- Test results are often not convincing and often not comparable because of the undefined and inconstant composition of the serum.
- Risk of a contamination with bacteria, fungi, mycoplasm and virus from serum.
- Risk of a contamination with TSE-agents (transmissible spongiform encephalopathy).
- Possibility of a contamination of the end product with serum proteins or pyrogens.
- Time-consuming cleaning of the end products from culture media containing serum.
- Availability and costs of the serum.

The Serumfree Cell Culture:

Because of the numerous disadvantages of the cell culture with nutrient media containing serum, tests have been made for quite some time to establish cell cultures under serumfree conditions.

Advantages of a Serumfree Cell Culture:

- Lower risk with regard to a contamination with bacteria, fungi or virus.
- Better defined and reproducible formulations allow more convincing and comparable research results.
- Time-consuming batch tests are not necessary.
- Elimination of a source for possible infectious agents (viruses, prions).
- Facilitation of the cleaning of the end products.
- Fulfilment of legal conditions for the production of medical products.
- Reduction of contaminations of the end products by culture residues.

Definitions:

Serumfree Media:

Serumfree media could be used without any supplementation or addition of Serum / FCS. In some compositions defined and purified components or subfractions of serum (e.g. Albumin, Transferrin or Insulin) could be contained or protein hydrolysates or gland extracts (BPE, BBE) are used.

Proteinfree Media:

Proteinfree media support the cell growth without any protein content. They contain higher concentrations of amino acids or herbal hydrolysates.

Chemically Defined Media:

Chemically defined media are completely free from any animal or human components. All components have a known chemically defined structure and composition. This leads to a very constant and stable definition with positive effects on quality, reproducibility and reduction of intercharge-variability.

Quality Assurance:

Each charge will only be produced with pretested premium raw material to ensure a stable and highest quality standard.

The quality of our pyrogenfree water is of highest purity with a conductance of 0,055 mS/cm and will be regularly examined as minimal quality variation will have detrimental effects on the cells in a serumfree culture.

All Panserin-charges will not be released until the quality-control process are finished and the required specifications have been acquired.

PANsys3000 – PANsys4000

In series of tests for many years PAN-Biotech has developed, optimized and successfully marketed a number of serumfree media for many different cell types. The development and optimization of serumfree media takes a lot of time and expenditure. Several months or even years can often pass until the release of a new product. PAN-Biotech used a very different approach for the development of serum-free media. For this purpose the new and fully automated cell culture systems from PAN-Systech – a subsidiary of PAN-Biotech – were used. PAN-Systech came about as the technology spin-off of PANBiotech GmbH - a long-established and well-known German biotechnology company. Today, PAN-Systech GmbH is a major producer of high tech products that are sold and valued worldwide. PAN-Systech develops, produces and markets a broad palette of innovative biotechnological systems related to cell-culture and laboratory automation, including the newest applications of bio-process technology. With the fully-automated cell culture Systems of PANSystech pursues a completely different technology. With these systems we have succeeded in cultivating different samples simultaneously under identical conditions and under constant microscopic observation. By adding individual components res. by changing the concentration, a positive (improvement of growth) res. negative effect (inhibition of growth) can directly be measured. All relevant data can be stored and recalled later. Morphologic changes are identified immediately and evaluated depending on the media composition.

Detailed information concerning the automated cell culture systems PANsys3000 and PANsys4000 can be found at www.pan-systech.com

The automated cell culture system offers unique features and many advantages for the development of serumfree media:

- Reduction of the development periods by more convincing results.
- Adjusting the optimal concentration of individual substances.
- Comparison of the measurements of competitor products and recording the results.
- Culture conditions (temperature, CO2-fumigation) can be continuously changed at any time.
- Complete control of individual batches.

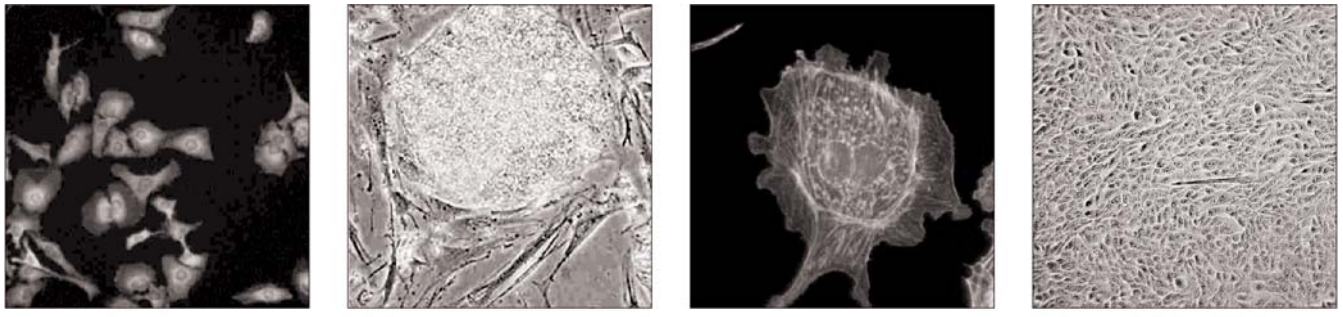
With these new technologies we were not only able to develop the ready-for-use nutrient solutions (PANSERINseries) but also a chemically defined serum-substitute (Panexin NTA and Panexin NTS) as well as a broad range of serum-free stemcell media (PowerStem-series).

Serumfree nutrient media without animal or human components resp. proteinfree media are also already available from PAN-Biotech.

Due to the modern and unique technology for the development of serumfree media of the 2nd-Generation in many cases an adaption of the cells to the serumfree culture is not necessary; with critical cells (primary cells) the cells slowly adapt to the serumfree conditions with the help of our detailed records and protocols. We will be pleased to support you with our expertise.

PANsys3000

PANsys4000



Cells cultivated in PANsys-Systems

Product Numbers

Chemically defined Serum-Substitute

Panexin NTA	100 ml 500 ml	P04-95700 P04-95750
Panexin NTS	100 ml 500 ml	P04-95800 P04-95850
Panexin BMM	100 ml	P04-951SA2

Serum-free „HighEfficiency“ Media

PANSERIN 401	100 ml 500 ml	P04-710401M P04-710401
PANSERIN 604	100 ml 500 ml	P04-710604M P04-710604
PANSERIN 293A	100 ml 500 ml	P04-710608M P04-710608
PANSERIN 293S	100 ml 500 ml	P04-710609M P04-710609
PANSERIN H4000	100 ml 500 ml	P04-714000M P04-714000
PANSERIN C6000	100 ml 500 ml	P04-716000M P04-716000
PANSERIN H8000	100 ml 500 ml	P04-718000M P04-718000
PANSERIN T3	100 ml 500 ml	P04-710110 P04-710100
PANSERIN ProVero	100 ml 500 ml	P04-710613M P04-710613
PANSERIN S2	100 ml 500 ml	P04-710210 P04-710200
SPODOPAN	100 ml 500 ml	P04-850100 P04-850500
Endopan 300 SL ready-to-use Endopan 300 SL kit	500 ml 500 ml	P04-00650 P04-0065K

Other Serum-free Media

PANSERIN 411	100 ml 500 ml	P04-710411M P04-710411
PANSERIN 411S	500 ml 1000 ml	P04-7411S1 P04-71411S
PANSERIN 412	100 ml 500 ml	P04-710412M P04-710412
PANSERIN 413 with one supplement	500 ml	P04-710413
PANSERIN 416 with one supplement	500 ml	P04-710416
PANSERIN 604ST	500 ml	P04-604ST
PANSERIN 604SPF	500 ml	P04-604SPF
PANSERIN 701	100 ml 500 ml	P04-710701M P04-710701
PANSERIN 801 with 6 supplements	500 ml	P04-710801
PANSERIN PX10	500 ml	P04-710PX10
PANSERIN PX40	500 ml	P04-710PX40

Serum-free Stemcell Media

PowerStem ESPro 1 with LIF	100 ml Kit 500 ml Kit	P04-7701K P04-77010K
PowerStem ESPro 1 without LIF	100 ml Kit 500 ml Kit	P04-7751K P04-77510K
PowerStem ESPro 2 with LIF	100 ml Kit 500 ml Kit	P04-7702K P04-77020K
PowerStem ESPro 2 without LIF	100 ml Kit 500 ml Kit	P04-7762K P04-77620K
PowerStem HE1	100 ml Kit 500 ml Kit	P04-7711K P04-77110K
PowerStem HE2	100 ml Kit 500 ml Kit	P04-7712K P04-77120K
PowerStem EST	100 ml Kit 500 ml Kit	P04-77210K P04-77250K
PowerStem MSC1	100 ml Kit 500 ml Kit	P04-77310K P04-77350K
PowerStem HPSC	100 ml Kit 500 ml Kit	P04-77410K P04-77450K
PowerStem PEC1 ready-to-use PowerStem PEC1 kit	500 ml 500 ml Kit	P04-777500 P04-77750K

PANEXIN NTA

PANEXIN NTA is a complete chemically defined serum substitute for the cultivation of adherent cells under serumfree conditions. PANEXIN NTA is developed with a unique technology and contains a special 3-dimensional substance release system (3D-SRS) for an optimal support of cells with nutrients and growth stimulants.

The ready to use, sterile solution is added to the culture medium in a final concentration of 10%. It supports the adherent growth of many cell types in an optimum manner.

Composition:
PANEXIN NTA contains purified proteins, lipids, salts, amino acids, trace elements, attachment factors and hormones in an optimized formulation and a new 3-dimensional substance release system (3D-SRS). PANEXIN NTA contains no growth factors, undefined hydrolysates or peptones.

Suitability:
PANEXIN NTA is suitable for the cultivation of a variety of adherent cells under serum-free culture conditions.

Special Advantages:
It has been shown for many cell lines that PANEXIN NTA can fully replace FBS. Due to selected and pretested raw materials PANEXIN NTA batches are very homogeneous. Therefore the complex batch testing known from FBS can be omitted with the use of PANEXIN NTA. In addition, there is no need to change the previously used basal medium. PANEXIN NTA is completely chemically defined and contains no undefined peptones or hydrolysates. Therefore, the interpretation of results from studies on effects of individually added growth factors is easier and more reliable in serum-free conditions. For cell lines which require specific growth factors these should be added in a concentration as previously used.

Instructions for Use :
In many cases a serum-free cultivation can be done without complex adaptation steps for adherent growing cell lines such as HEK293, CHO, L929, 3T3A.

- Thaw PANEXIN NTA in a water bath at 37 °C. Please avoid repeated freeze-thaw cycles!
- Trypsinate adherent cells as usual (e.g. 0.25% trypsin solution). Once the cells have become round and detach from the surface (the process can be speeded at 37 °C) inactivate trypsin with trypsin inhibitor.
- Rinse the cells with DPBS (without Mg^{++}/Ca^{++}) and centrifuge, sterile remove supernatant.
- Resuspend cells in basal medium (e.g. RPMI 1640, DMEM or other) and count.
- Add 10% sterile PANEXIN NTA to basal medium to replace FBS.
- Add the cell suspension to the basal medium supplemented with PANEXIN NTA.
- Initial seeding density 5,000 – 20,000 cells/cm^2.
- Incubate the cells in the usual way in a CO_2 incubator at 37 °C.

Depending on the cell type, the optimal PANEXIN NTA concentration can vary from 5% to 15%, comparable to FBS concentrations used. The optimal PANEXIN NTA concentration should be determined for each cell line. The tests should be started at a PANEXIN NTA concentration of 10% as in most cells the best results were obtained with this concentration.

As a basal medium you may use classical standard media such as RPMI 1640, DMEM (high or low glucose), DMEM/F12, IMDM and so on. Make sure that L-glutamine is present in sufficient quantity (possibly supplement glutamine).

Depending on the cell type, some differences in morphology or proliferation rate may be observed with various standard media. Many applications were performed with DMEM or DMEM/F12 for adherent cells. With these combinations very good growth stimulation was achieved in a range of 5% to 15% PANEXIN NTA.

For more demanding cells an adaptation to PANEXIN NTA may be necessary.

Adaptation instructions for PANEXIN NTA:
A major precondition for a successful transition from serumcontaining to serum-free culture are vital cells (trypan blue exclusion staining, viability >90%), which should be harvested in the logarithmic growth phase.

- Harvest cells as usual.
- Supplement your basal medium with 10% PANEXIN NTA = MedPAN
- The final solution is stable for at least 4 weeks at 4 °C
- Supplement your basal medium with 10% FBS = MedFBS

1) 75% MedFBS : 25% MedPAN

- Seed cells at 5,000 – 20,000 cells/cm^2.
- Observe cells under a microscope, at about 90% confluence, passage the cells for another 2-3 passages.

If normal growth is obtained transfer cells into:

2) 50% MedFBS : 50% MedPAN

- Seed cells at 5,000 – 20,000 cells/cm^2.
- Observe cells under a microscope, at about 90% confluence, passage the cells for another 2-3 passages.

If normal growth is obtained transfer cells into:

3) 25% MedFBS : 75% MedPAN

- Seed cells at 5,000 – 20,000 cells/cm^2.
- Observe cells under a microscope, at about 90% confluence, passage the cells for another 2-3 passages.

If normal growth is obtained transfer cells into:

4) 100% MedPAN

- Seed cells at 5,000 – 20,000 cells/cm^2.
- Observe cells under a microscope.

PANEXIN NTA

For some cells an adaptation to serum-free conditions is difficult to reach or even impossible. The following measures may help to facilitate a successful adaptation:

- Reseeding with a higher cell amount (about 2x to 4x of the usual cell density).
- Addition of growth factors (if known, which factors have a positive effect on the relevant cells).
- Coating the culture dishes or flasks with attachment factors (e.g. fibronectin, laminin, collagen, gelatine, or other).
- Change the basal medium. Note: A change of the basal medium to a richer or more complex formulation may be all that is needed to achieve growth in serum free condition.

References:

a) Hashimoto J et al. (2006) Regulation of Proliferation and Chondrogenic Differentation of Human Mesenchymal Stem Cells by Laminin-5 (Laminin-332). Stem Cells 24:2346

b) Traeger T et al. (2008) Detrimental Role of CC Chemokine Receptor 4 in Murine Polymicrobial Sepsis. Infection and Immunity 11:5285

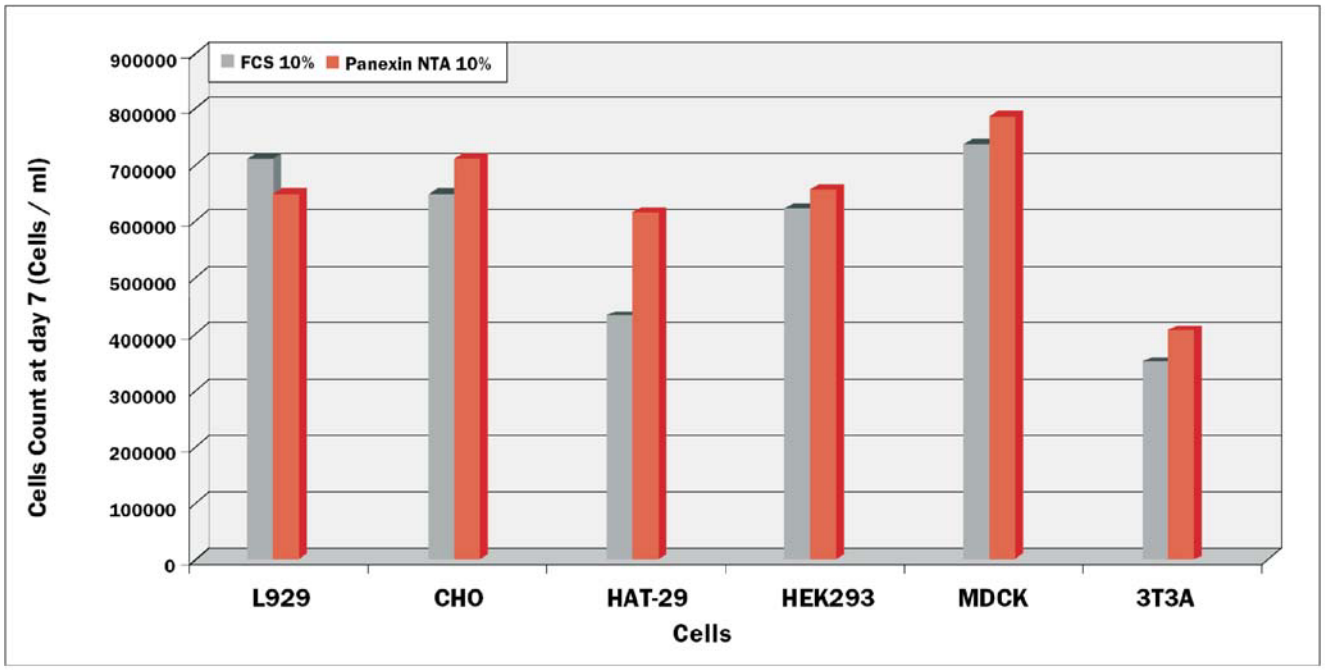
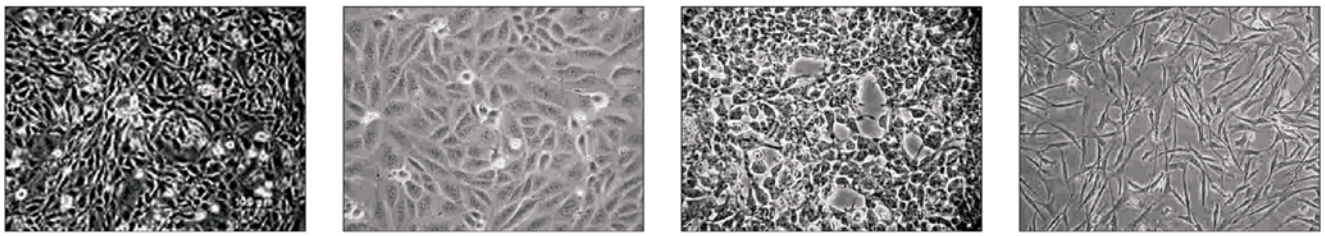


Fig. 1: Efficiency and Growth Stimulation of PANEXIN NTA compared to FBS (each 10% in DMEM/F12)



CHO Cells 10% PANEXIN NTA in DMEM/F12

MDCK Cells 10% PANEXIN NTA in DMEM/F12

HEK 293 Cells 10% PANEXIN NTA in DMEM/F12

Primary human Fibroblasts 10% PANEXIN NTA in DMEM/F12

Fig. 2: Different Cell Lines in DMEM/F12 with 10% PANEXIN NTA

Panexin NTA	100 ml 500 ml	P04-95700 P04-95750

PANEXIN NTS

PANEXIN NTS is a complete chemically defined serum substitute for the cultivation of suspension cells under serum-free conditions. PANEXIN NTS is developed with a unique technology and contains a special 3-dimensional substance release system (3D-SRS) for an optimal support of cells with nutrients and growth stimulants. The ready to use, sterile solution is added to the culture medium in a final concentration of 10%. It supports the growth of many cell types in an optimum manner.

Composition:
PANEXIN NTS contains purified proteins, lipids, salts, amino acids, trace elements, and hormones in an optimized formulation and a new 3-dimensional substance release system (3D-SRS). PANEXIN NTS contains no growth factors, undefined hydrolysates or peptones.

Suitability:
PANEXIN NTS is suitable for the cultivation of a variety of non-adherent suspension cells under serum-free conditions.

Special Advantages:
PANEXIN NTS can be used for many cell lines to replace FBS. Due to selected and pretested raw materials PANEXIN NTS batches are very homogeneous. Therefore the complex batch testing known from FBS can be omitted with the use of PANEXIN NTS. In addition, there is no need to change the previously used basal medium. PANEXIN NTS is completely chemically defined and contains no growth factors, undefined peptones or hydrolysates. Therefore, the interpretation of results from studies on effects of individually added growth factors is easier and more reliable in serum-free conditions. For cell lines which require specific growth factors, these should be added in a concentration as previously used.

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Instructions for Use :
In many cases a serum-free cultivation can be done without complex adaptation steps (suspension cell lines such as HL60, K562, as well as hybridoma cells).

- Thaw PANEXIN NTS in a water bath at 37 °C. Please avoid repeated freeze-thaw cycles!
- Non-adherent cells (e.g. SP2) can be directly transferred into the nutrient solution (e.g. RPMI 1640, IMDM) supplemented with 10% PANEXIN NTS.
- Initial seeding density $5x10^4$ - $1x10^5$ cells/ml.

Depending on the cell type the optimal PANEXIN NTS concentration can vary from 5-15%, comparable to FBS concentrations used previously. The optimal PANEXIN NTS concentration should be determined for each cell line. The tests should be started at a PANEXIN NTS concentration of 10% as for most cells the best results were obtained with this concentration.

As a basal medium you can use classical standard media such as RPMI 1640, DMEM (high or low glucose), DMEM/F12, IMDM and so on. Make sure that L-glutamine is present in sufficient quantities (possibly supplement glutamine).

Depending on the cell type, some differences in morphology or proliferation rate could be observed with the various standard media.Many applications were performed with RPMI 1640 and IMDM for non-adherent cells. With these combinations very good growth stimulation was achieved with 5-15% PANEXIN NTS.

For more demanding cells an adaptation to PANEXIN NTS may be necessary.

Adaptation instructions for PANEXIN NTS:
A precondition for a successful transition are vital cells (trypan blue exclusion staining, viability >90%), which should be harvested in the logarithmic growth phase.

- Harvest cells as usual.
- Supplement your basal medium with 10% PANEXIN NTS = MedPAN
- The final solution is stable for at least 4 weeks at 4 °C.
- Supplement your basal medium with 10% FBS = MedFBS

1) 75% MedFBS : 25% MedPAN

- Seed cells at $5x10^4$ – $1x10^5$ cells/ml (e.g. T25, 10 ml).
- Observe cells under a microscope, in the case of good proliferation (e.g. cell count > $1x10^6$ cells/ml), passage the cells for another 2 - 3 passages.

If normal growth is obtained transfer cells into:

2) 50% MedFBS : 50% MedPAN

- Seed cells at $5x10^4$ – $1x10^5$ cells/ml (e.g. T25, 10 ml).
- Observe cells under a microscope, in the case of good proliferation (e.g. cell count > $1x10^6$ cells/ml), passage the cells for another 2 - 3 passages.

If normal growth is obtained transfer cells into:

3) 25% MedFBS : 75% MedPAN

- Seed cells at $5x10^4$ – $1x10^5$ cells/ml (e.g. T25, 10 ml).
- Observe cells under a microscope, in the case of good proliferation (e.g. cell count > $1x10^6$ cells/ml), passage the cells for another 2 - 3 passages.

If normal growth is obtained transfer cells into:

4) 100% MedPAN

- Seed cells at $5x10^4$ – $1x10^5$ cells/ml (e.g. T25, 10 ml).
- Observe cells under microscope.

PANEXIN NTS

For some cells an adaptation to serum-free conditions is difficult to reach or even impossible.

The following measures may help to facilitate a successful adaptation:

- Reseeding with a higher cell amount (about 2x to 4x of the usual cell density).
- Change of basal medium. Note: A change of the basal medium to a richer or more complex formulation may be all that is needed to achieve growth in serum-free condition.

References:

a) Breitbach K et al. (2009) Caspase-1 Mediates Resistance in Murine Melioidosis. Infection and Immunity 4:1589

b) Into T et al. (2008) Regulation of MyD88-Dependent Signaling Events by S Nitrosylation Retards Toll-Like Receptor Signal Transduction and Initiation of Acute-Phase Immune Responses. Molecular and Cellular Biology 4:1338

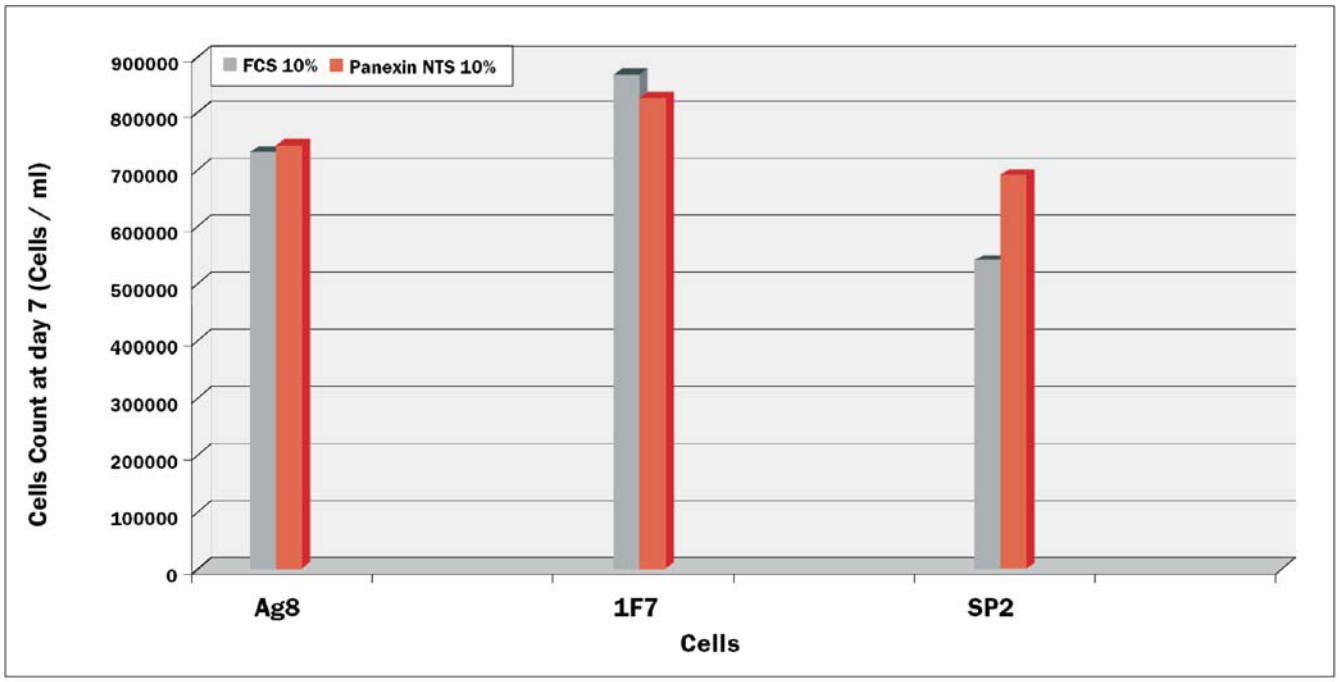
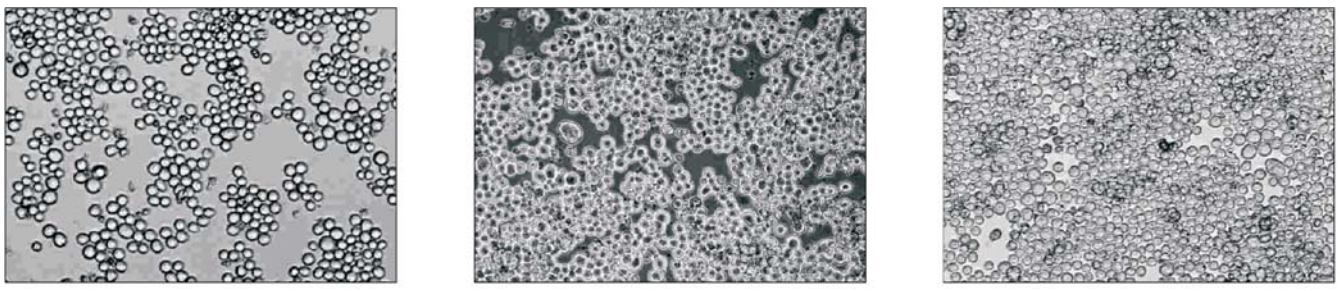


Fig. 1: Efficiency and Growth Stimulation of PANEXIN NTS compared to FBS (each 10% in RPMI)



Ag8 Cells
10 % PANEXIN NTS in RPMI

1F7 Cells
10 % PANEXIN NTS in RPMI

SP2 Cells
10 % PANEXIN NTS in RPMI

Fig. 2: Different Cell Lines in RPMI with 10% PANEXIN NTS

Panexin NTS	100 ml 500 ml	P04-95800 P04-95850

PANEXIN BMM

Panexin BMM is a chemically defined serum substitute for the cultivation of macrophages from mouse bone marrow (murine bone marrow derived macrophages, BMM) under serum-free conditions. The ready-to-use sterile solution in a final concentration of 5 % is added to the basalmedium RPMI 1640, supplemented with 50 µM Mercaptoethanol and 2 ng/ml GM-CSF mur. rec.

Composition:
Panexin BMM contains purified proteins, lipids, salts, amino acids, trace elements, attachment factors and hormones in an optimized formulation. It contains no growth factors, undefined hydrolysates or lysates (e. g. Peptones).

Suitability:
Panexin BMM has been developed for the generation of murine macrophages from bone marrow under serum-free conditions. This achieves standardized conditions and reproducible results.

Particular benefits:
Panexin BMM allows the generation of murine macrophages from bone marrow under standardized serum-free conditions. The results will be more comparable, as undefined components – like in serum cultures – are eliminated. In Panexin BMM matured macrophages will show excellent attachment capabilities.

Application:
For the production of complete serum-free medium, add 5 % Panexin BMM to RPMI 1640, supplemented with 50 µM Mercaptoethanol and 2ng/ml GM-CSF (murine, rec.). After the isolation of the bone marrow from a mouse, a single cell suspension will be achieved through frequent pipetting. Then centrifuge the cells at 200 x g for 10 minutes. Resuspend the isolated cells in the serum-free medium and seed the cells in three 75 mm^2 culture bottles within 20 ml of medium. The incubation is done at 37 °C and 5 % CO2 fumigation in the incubator. The feeding of the cells should be done on day 5 and 7 by an exchange of 10 ml of medium with fresh medium. After 10 days, the cells can be harvested.

References:
Kristin Eske, Katrin Breitbach, Jens Köhler, Patimaporn Wongprompitak and Ivo Steinmetz (2008).
Generation of murine bone marrow derived macrophages in a standardised serum-free cell culture system, Journal of Immunological Methods.

Panexin BMM	100 ml	P04-951SA2

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PANSERIN 401

Panserin 401 is a complete ready to use medium for the serum-free cultivation of a multitude of adherent and nonadherent cells.

Storage conditions:
Storage: +2 °C to +8 °C
Stability: 10 months
Filling: 100 ml, 500 ml, other fillings on request

Composition:
Based on Iscove s MEM, trace elements, albumin, cholesterol, soya lipids and vitamins were added to the medium. It does not contain any growth or attachment factors.

Suitability:
Panserin 401 is a multi-purpose medium suitable for a variety of cells. In Panserin 401 adherent as well as nonadherent cells can be cultivated. As the medium contains no growth factors there is a possibility to investigate the effects of specific growth factors added to the cell culture. Panserin 401 does not contain any attachment factors. With some cell types a pre-treatment of the cell culture vessels with gelatine, collagen, poly-D-lysine or fibronectin may support or enable a culture under serum-free conditions. Please note that a coating may be especially important with low seeding densities.

With every adaption to serum-free media, changes of the cells should be taken into consideration. These changes may concern morphology, karyotype, surface markers and so on. Thus cells in serum-free medium may not be identical with those from cultures containing serum in which they originated (selection).

Among others the following cells have been cultivated successfully:

- Hybridoma
- Lymphocytes
- Macrophages
- Fibroblasts
- Melanocytes
- Carcinoma cells
- HEK-cells
- HeLa-cells
- CHO-cells

Instructions for use:
In many cases the switch from serum-containing to serumfree cultivation can be done without any special adaption procedures. For those cells which do not tolerate an immediate switch we recommend a primary culture with serum containing medium and a stepwise reduction of medium towards a serum-free cultivation. we can

PANSERIN 401

provide you with an adaption protocol for many different cell types.
This stepwise adaption will also be supported by higher cell seeds or using a lowered serum concentration after attachment of the cells in medium containing a higher amount of serum.

For the successful transfer into serum-free cultivation the vitality of the cells is an important factor. Thus the cells should be transferred in the logarithmic growth phase. According to our experience the transfer in the logarithmic growth phase will have higher prospects of success.

In adherent cells it should be assured that - if trypsin is used for detachment - the enzyme is completely washed out or is inactivated by trypsin-inhibitors because there is no trypsin inactivating effect of FBS; use trypsin-inhibitor to stop trypsin activity. In some cases of very sensitive cells it could be also reasonable to do the stepwise adaption and dilution not only with serum but also in the medium which has been used so far.

Panserin media were developed to support cell growth without the use of serum. Thus the all-round version (Panserin 401) does not contain any growth factors.

Studies on the effect of externally added growth factors will be more valid. For cells which are dependent on specific growth factors these factors should be added in the required concentrations.

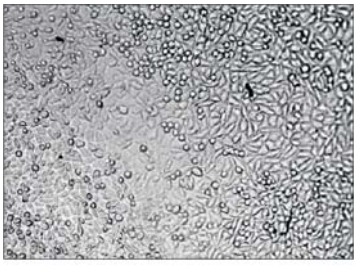
References:

a) Pilar S et al. (2002) Contribution of CD3y to TCR regulation and signaling in human mature T lymphocytes. International Immunology 11:1357

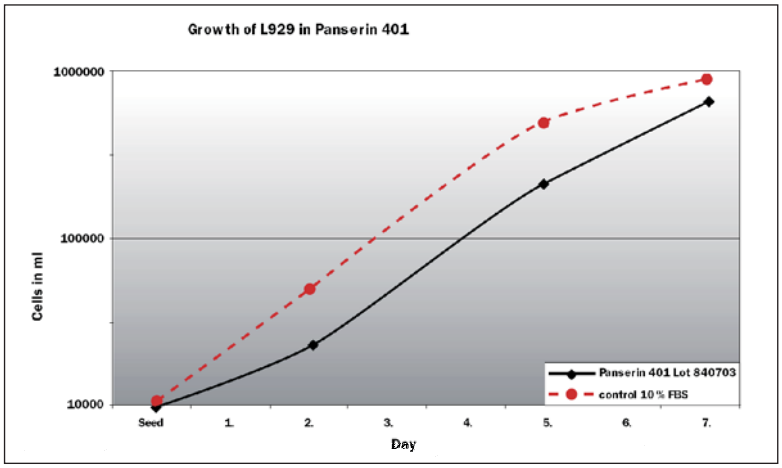
b) Toptan T et al. (2010) Rhadinovirus vector-derived human telomerase reverse transcriptase expression in primary T cells. Gene Therapy 17:653

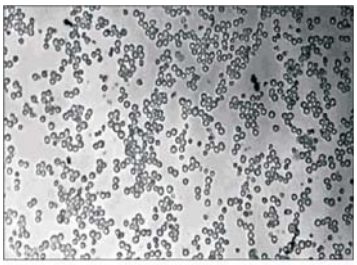
c) Martin F et al. (2005) Lentiviral vectors transcriptionally targeted to hematopoietic cells by WASP gene proximal promotor sequences. Gene Therapy 12:715

d) Montzka K et al. (2010) Expansion of human bone marrowderived mesenchymal stromal cells: serum-reduced medium is better than conventional medium. Cytotherapy 5:587 For more references see www.pan-biotech.de.

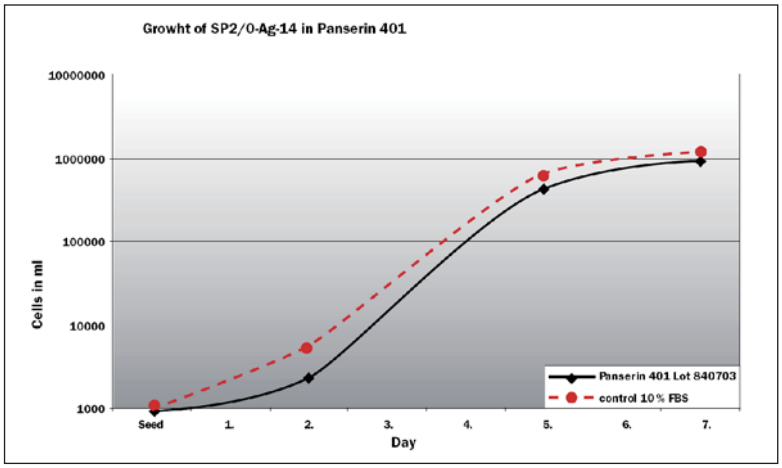


L 929 in Panserin 401 without prior adaption





SP2/0-Ag-14 in Panserin 401 without prior adaption



PANSERIN 401	100 ml 500 ml	P04-710401M P04-710401

PANSERIN 604

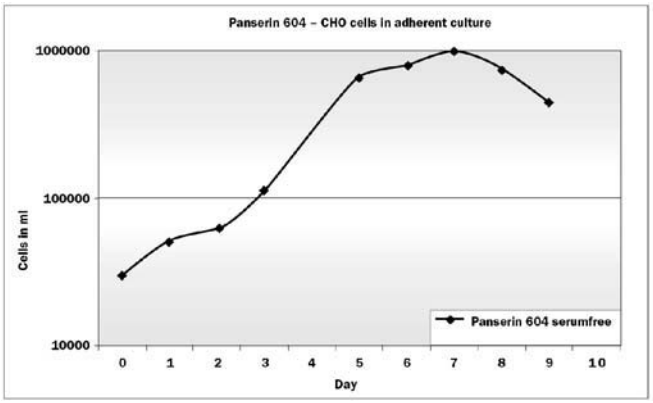
Panserin 604 is a complete medium ready for use for the cultivation of transfected and non-transfected CHO-cells (Chinese Hamster Ovary) in an adherent culture.

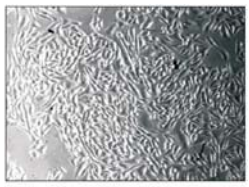
Composition:
Based on Glasgow medium, trace elements, albumin, cholesterol, soya-lipids, vitamins hormones and attachment factors were added to the medium.

Suitability:
Cultivation of transfected and non-transfected CHO-cells in adherent culture (e.g. roller cultures)

Special Advantages:
Highly enriched medium for the fast growth and culture of adherent CHO-cells.

Instructions:
In many cases the switch from serum-containing medium to Panserin 604 can be done without any special adaption procedures.
For those cells which do not tolerate an immediate switch we recommend a primary culture with Panserin 604 supplemented with serum and than a stepwise reduction of serum towards a pure Panserin 604 cultivation.
This stepwise adaption will also be supported by higher cell seeds or by using the lowered serum concentration after attachment of the adherent cells.
For the successful transfer into serumfree cultivation the vitality of the cells is an important factor. Thus the cells should be transferred in the logarithmic growth phase. According to our experience a transfer within the stationary growth phase will have lower prospects of success. In adherent cells it should be assured that – if trypsin is used for detachment – the enzyme is completely washed out or is inactivated by trypsin-inhibitors in order for the serum to have no neutralizing effect.





CHO cells in Panserin 604

PANSERIN 604	100 ml 500 ml	P04-710604M P04-710604

PANSERIN 293A

Panserin 293A is a complete ready to use medium for the serum-free cultivation of HEK293 cells (Human Embryonic Kidney) in adherent culture.

Storage conditions:
Storage: +2 °C to +8 °C
Stability: 1 year
Filling: 100 ml, 500 ml, other fillings on request

Composition:
Based on DMEM additional trace elements, albumin, cholesterol, soy lipids, vitamins and hormones have been added to the medium.

Suitability:
Panserin 293A is a particularly enriched medium optimized for the growth of HEK293 cells in adherent culture.

Introduction for use:
A switch from serum-containing medium to Panserin 293A is often possible without adaptation. For those clones which do not tolerate an immediate switch we recommend a primary culture with serum containing medium and a stepwise reduction of serum towards a serum-free cultivation with Panserin 293A.
The efficient serum-free cultivation is supported by higher seeding densities. For the successful transfer into serumfree cultivation the vitality of the cells is an important factor. Thus the cells should be transferred in the logarithmic growth phase. According to our experience the transfer within the logarithmic growth phase will have higher prospects of success.
During the cell transfer it should be assured that - if trypsin is used for detachment - the enzyme is completely washed out or is inactivated by trypsin-inhibitors due to an absence of any trypsin-neutralizing effect by FBS.

PANSERIN 293A	100 ml 500 ml	P04-710608M P04-710608

PANSERIN 293S

Panserin 293S is a complete ready to use medium for the serum-free cultivation of HEK293 cells (Human Embryonic Kidney) in suspension culture.

Storage conditions:
Storage: +2 °C to +8 °C
Stability: 1 year
Filling: 100 ml, 500 ml, other fillings on request

Composition:
Based on DMEM/F12 medium additional trace elements, cholesterol and herbal hydrolysates have been added. Panserin 293S does not contain any proteins or components of animal or human origin.

Suitability:
Panserin 293S is a particularly enriched medium optimized for the growth of HEK293 cells in suspension culture and quickly provides high cell densities. Due to its protein-free formulation the purification of final products (recombinant proteins, viruses) from the cell culture is more convenient and economic. Cell clustering - often seen in serum-free suspension cultures – will be reduced significantly in Panserin 293S.

Introduction for use:
A switch from adherent serum-containing medium to Panserin 293S is often possible without adaptation. For those clones which do not tolerate a direct switch, we recommend a primary culture with serum containing medium and a stepwise reduction of serum towards a serum-free cultivation with Panserin 293S.

The efficient serum-free cultivation is supported by higher seeding densities. For the successful transfer into serumfree cultivation the vitality of the cells is an important factor. Thus the cells should be transferred in the logarithmic growth phase. According to our experience the transfer within the logarithmic growth phase will have higher prospects of success.

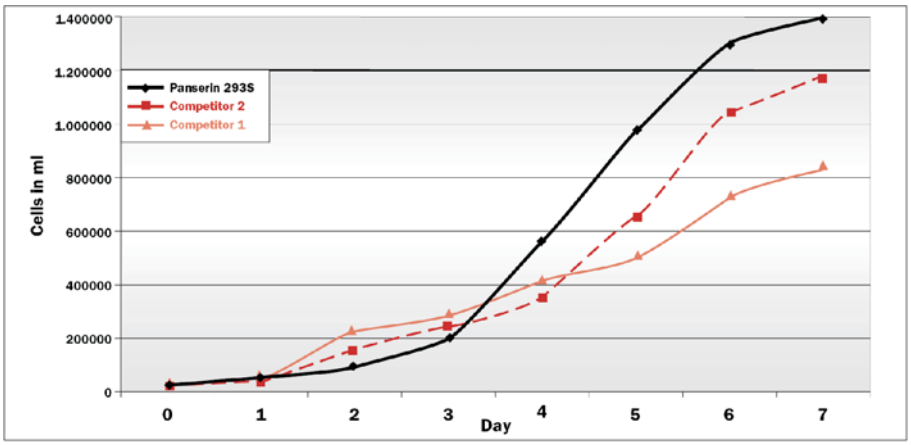


Fig.: Growth of HEK293 in Panserin 293S

PANSERIN 293S	100 ml	P04-710609M
	500 ml	P04-710609

PANSERIN H4000

Panserin H4000 is a protein-free ready to use medium for an optimized growth of myeloma and hybridoma-cell lines in suspension culture for the production of monoclonal antibodies. Panserin H4000 is suitable for spinner cultures, roller bottles and tissue culture bioreactors

Storage conditions:
Storage: +2 °C to +8 °C
Stability: 1 year
Filling: 100 ml, 500 ml, other fillings on request

Composition:
Panserin H4000 consists of a balanced mixture of salts, amino acids, vitamins, trace elements, hormones and is enriched with selected herbal hydrolysates for an optimized growth of myeloma and hybridoma cell lines. As Panserin H4000 is free of animal or human components it is predestined for the use in sensitive production areas (e.g. production of diagnostic or therapeutic tools) where safety requirements prohibit the use of human or animal components.

Suitability:
Cultivation of myeloma and hybridoma cell lines for the production of monoclonal antibodies.

Special Advantages:
The formulation of the protein-free Panserin H4000 with a low concentration of plant hydrolysates enables a high cell yield in combination with excellent production rates of monoclonal antibodies. The ready to use protein-free medium allows easy handling and therefore reduces contamination risks and ensures for an easy and economic

PANSERIN H4000

Instructions for use:
Adaption to a protein-free culture

Most hybridoma cell lines can be directly transferred from a serum containing culture into the protein-free suspension culture. It should be noted here that the seeding density should be at least 1-3 x 10^5 cells. For cholesterol-dependent cells (e.g. X63AG8.653) use Panserin H8000 (cat.-no.: P04-718000).

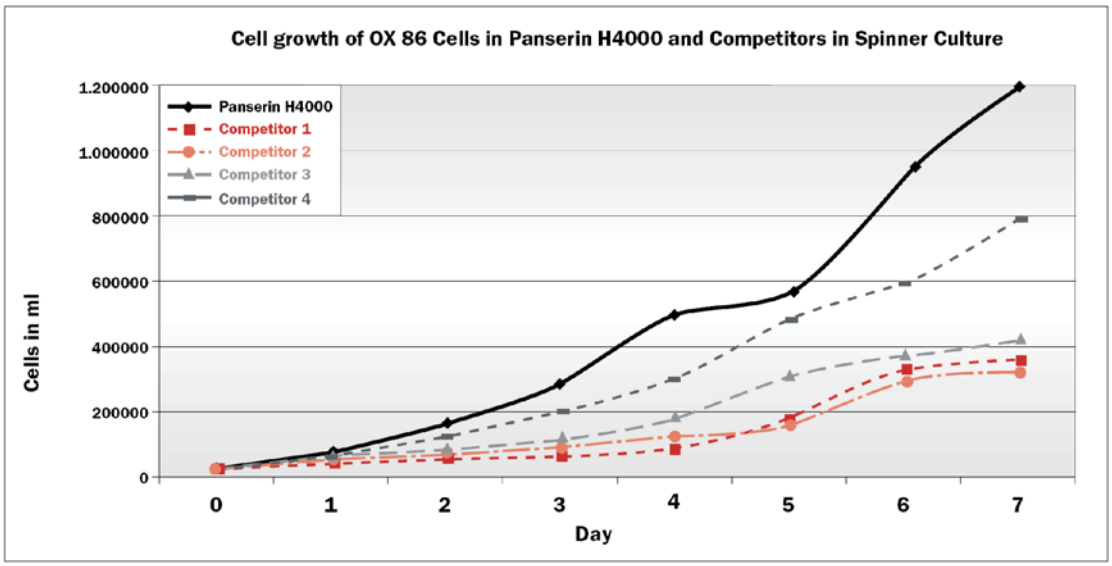
Direct adaptation to Panserin H4000:

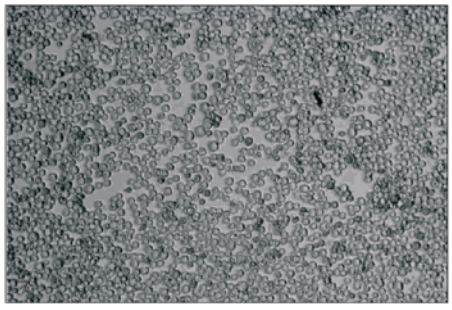
- Use cells from a serum-containing culture (e.g. RPMI 1640 with 10% FBS) in the log-phase (80% of maximum cell density).
- Determine cell count and control vitality with trypan blue exclusion staining.
- Seed approx. 1-3 x 10^5 cells/ml in preheated Panserin H4000.
- Incubate the cells in an incubator at 37 °C and 5% CO_2.
- Once the cells have reached approx. 80% of the maximum density transfer the cells into fresh Panserin H4000. Initially maintain high seeding densities until the cells have adapted to the protein-free culture.
- When the growth rate is comparable to the serum containing culture the cells should be transferred into fresh Panserin H4000 every 3-4 days.
- If the growth rate is not sufficient or the maximal cell densities are not reached, perform the described indirect adaption as described below.

Indirect adaptation to Panserin H4000:

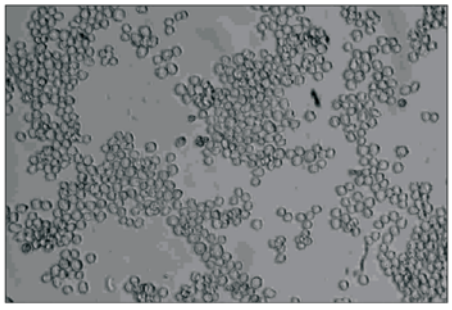
- Use cells from a serum-containing culture (e.g. RPMI 1640 with 10% FBS) in the log-phase (80% of maximum cell density).
- Determine cell count and control vitality with trypan blue exclusion staining. • Seed approx. 1-3 x 10^5 cells/ml in preheated Panserin H4000 with 5% FBS.
- Incubate the cells in an incubator at 37 °C and 5% CO_2.
- Once the cells have reached approx. 80% of the maximum density transfer the cells into fresh Panserin H4000 with 2% FBS.
- During the next splitting step use Panserin H4000 with 1% FBS and finally use Panserin H4000 with 0.1% FBS (same steps as mentioned above).

When the growth rate is comparable to the serum-containing culture the cells should be transferred into fresh Panserin H4000 without any additional FBS every 3-4 days.





SP2/0-Ag-14 in Panserin H4000



OX-86 in Panserin H4000

PANSERIN H4000	100 ml 500 ml	P04-714000M P04-714000

PANSERIN C6000

Panserin C6000 is a protein-free ready to use medium for an optimized growth of CHO-cells (Chinese Hamster Ovary) and their recombinant derivates in suspension culture. These cells are often used for the production of recombinant proteins for diagnostic or therapeutic purposes. Panserin C6000 is suitable for spinner cultures, roller bottles and tissue culture flasks and bioreactors.

Storage conditions:
Storage: +2 °C to +8 °C
Stability: 1 year
Filling: 100 ml, 500 ml, other fillings on request

Composition:
Panserin C6000 consists of a balanced mixture of salts, amino acids, vitamins, trace elements, hormones and is enriched with select herbal hydrolysates for an optimized growth of CHO-cells in suspension culture. As Panserin C6000 is free of animal or human components it is predestined for the use in sensitive production areas (e.g. production of diagnostic or therapeutic tools) where safety requirements prohibit the use human or animal components.

Suitability:
Protein-free cultivation of CHO-cells and their recombinant derivates in suspension culture for the production of recombinant proteins for diagnostics or therapeutic purposes.

Special Advantages:
The formulation of the protein-free Panserin C6000 with a low concentration of plant hydrolysates enables a high cell yield in combination with excellent production rates of recombinant proteins. The ready to use complete protein-free medium allows easy handling and therefore reduces contamination risks and ensures for an easy and economic purification of the final products in down-stream processes. Due to the optimized composition of Panserin C6000 the cells expand and grow in single-cell suspension with a very low tendency to form aggregates

Instructions for use:
Adaption to a protein-free culture

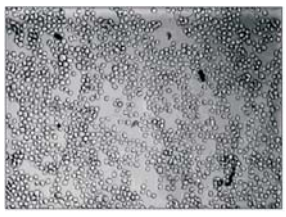
Most CHO-cells can be directly transferred from a serum containing adherent culture into the protein-free suspension culture. In most cases the stable suspension culture is developing within approx. 2 weeks.

Direct adaptation to Panserin C6000:

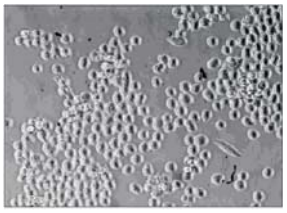
- Use adherent cells in the log phase of a serum containing culture (for example high glucose DMEM with 10% FBS).
- Remove serum containing medium with a pipette.
- Wash cell layer with DPBS without Ca/Mg.
- Cover cell layer with trypsin / EDTA (0.25%, 0.02%) (about 2 ml per T25 flask).
- Remove trypsin after about 1 minute.
- Incubate the cells until they show a round figure and detach from the surface (about 5 minutes).
- Transfer cells into DPBS and count the cell number.
- Seed 5 x 10^4 - 1 x 10^5 cells/ml in preheated Panserin C6000. Use flasks for suspension culture (e.g. greiner bio-one Code 690 190).
- Incubation at 37 °C and 5% CO_2 in an incubator.

For a few days the cells will stay in a lag-phase where no significant cell proliferation will occur. After about one week, however, the cells will be accustomed to the proteinfree medium and will have proliferated to 1x10^6 cells/ml.

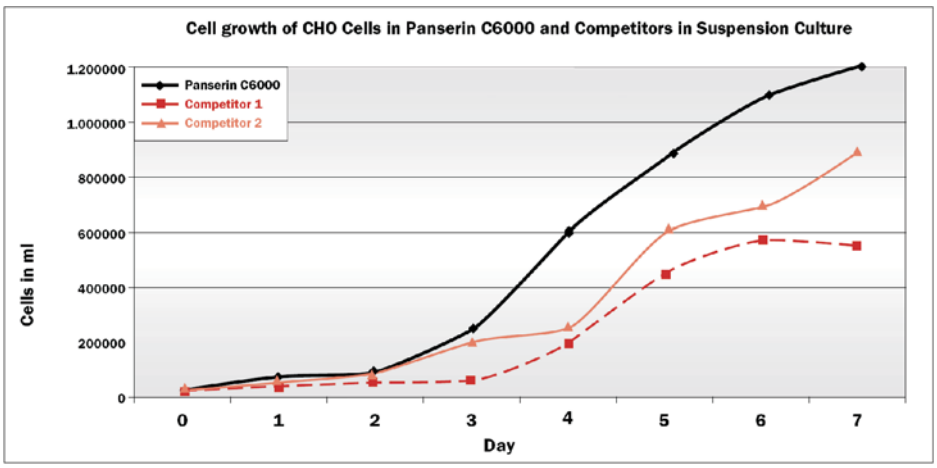
Transfer the cells every 3 to 4 days into fresh medium. (Seeding density 3-5 x 10^4 cells/ml). Generation time of CHO-cells in Panserin C6000: 16.5 h.



CHO cells in Panserin C6000



CHO cells in Panserin C6000



PANSERIN C6000	100 ml 500 ml	P04-716000M P04-716000

PANSERIN H8000

Panserin H8000 is a protein-free ready to use medium for an optimized growth of cholesterol-dependent myeloma and hybridoma cell lines in suspension culture for the production of monoclonal antibodies. Panserin H8000 is suitable for spinner cultures, roller bottles and tissue culture bioreactors.

Storage conditions:
Storage: +2 °C to +8 °C
Stability: 1 year
Filling: 100 ml, 500 ml, other fillings on request

Composition:
Panserin H8000 consists of a balanced mixture of salts, amino acids, vitamins, trace elements, hormones, bioavailable cholesterol and is enriched with selcted herbal hydrolysates for an optimized growth of cholesteroldependent myeloma and hybridoma cell lines.

Suitability:
Cultivation of cholesterol-dependent myeloma and hybridoma cell lines for the production of monoclonal antibodies.

Special Advantages:
The formulation of the protein-free Panserin H8000 with a low concentration of plant hydrolysates enables a high cell yield in combination with excellent production rates of monoclonal antibodies. As Panserin H8000 is free of animal or human components it is predestined for the use in sensitive production areas (e.g. production of diagnostic or therapeutic tools) where safety requirements prohibit the use of human or animal components. The ready-touse protein-free medium allows easy handling and therefore reduces contamination risks and ensures an easy and economic purification of final products in the downstream processing.

Instructions for use:
Adaption to a protein-free culture

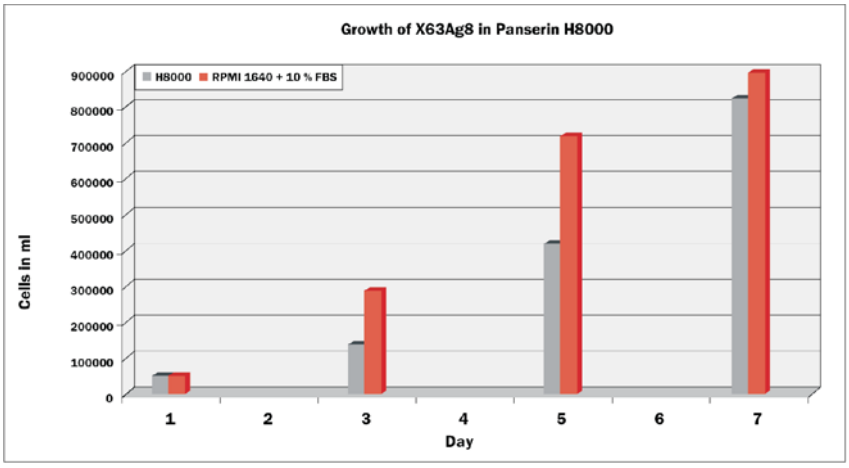
Most hybridoma cell lines can be directly transferred from a serum-containing culture into a protein-free suspension culture. It should be noted here that the seeding density should be at least 1-3 x 10^5 cells.

Direct adaptation to Panserin H8000:

- Use cells from a serum containing culture (e. g. RPMI 1640 with 10% FBS) in the log-phase (80% of the maximum cell density).
- Determine cell count and control vitality via trypan blue exclusion staining.
- Seed approx. 1-3 x 10^5 cells/ml in preheated Panserin H8000.
- Incubate the cells in an incubator at 37 °C and 5% CO_2
- Once the cells have reached approx. 80% of the maximum density transfer the cells into fresh Panserin H8000. Maintain high seeding densities until the cells have adapted to the protein-free culture.
- When the growth rate is comparable to the serum containing culture the cells should be transferred into fresh Panserin H8000 every 3-4 days.
- If the growth rate is not sufficient or maximum cell densities are not reached, perform the indirect adaption described below.

Indirect adaptation to Panserin H8000:

- Use cells from a serum containing culture (e. g. RPMI 1640 with 10% FBS) in the log-phase (80% of the maximum cell density).
- Determine cell count and control vitality via trypan blue exclusion staining.
- Seed approx. 1-3 x 10^5 cells/ml in preheated Panserin H8000 with 5% FBS
- Incubate the cells in an incubator at 37 °C and 5% CO_2
- Once the cells have reached approx. 80% of the maximum confluence transfer the cells into fresh Panserin H8000 with 2% FBS.
- During the next splitting step use Panserin H8000 with 1% FBS and finally use Panserin H8000 wit 0.1% FBS (same steps as mentioned above).
- When the growth rate is comparable to the serum containing culture the cells should be transferred into fresh Panserin H8000 without any additional FBS every 3-4 days.



PANSERIN H8000	100 ml 500 ml	P04-718000M P04-718000

PANSERIN T3

Panserin T3 is a ready to use serum-free complete medium for the cultivation of 3T3A cells in suspension culture.

Composition:
Panserin T3 is a fully defined serum-free complete medium. Based on Iscove´s MEM, this medium was supplemented with cholesterin, soylipids, albumin, vitamins and trace elements. It contains no growth and attachment factors.

Suitability:
Panserin T3 was developed for the serum-free cultivation of mouse fibroblasts (3T3A) in suspension.

Instructions for use:
A change from serum-containing medium to Panserin T3 is usually possible without special adaptation. This process is supported by higher cell seeding. Cells should be seeded in a density of about 105 cells/ml. It is important to use culture flasks for suspension culture. After several passages in serum-free culture at lower growth rates, the cells reach high growth rates. For the successful transfer of the cells in a serum-free culture the viability is an important factor. Therefore use cells from the logarithmic growth phase. From our experience, the cultivation of cells from the stationary phase will have lower chances of success.

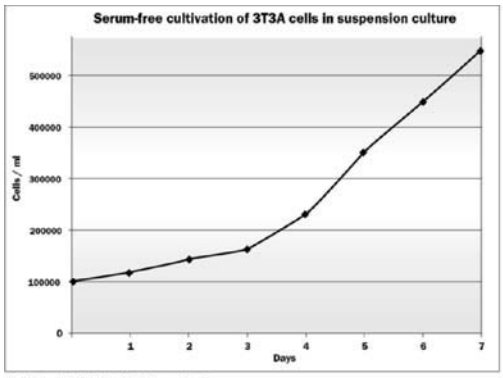


Fig. 1: Growth of 3T3A cells in Panserin T3

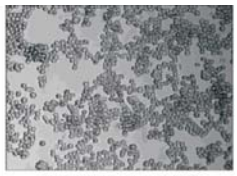


Fig. 2: 3T3A-cells in Panserin T3

PANSERIN T3	100 ml	P04-710110
	500 ml	P04-710100

PANSERIN ProVero

Panserin ProVero is a complete serum-free medium ready to use for the cultivation of Vero cells (kidney epithelial cells from African green monkey) in an adherent culture.

Composition:
Panserin ProVero is based on DMEM/F12. It contains trace elements, albumin, cholesterol, soya-lipids, vitamins, hormones and attachment factors.

Suitability:
Cultivation of Vero cells in adherent culture (e.g. roller cultures)

Special Advantages:
Highly enriched medium for the fast growth and culture of adherent Vero cells.

Instructions for use:
In many cases the switch from serum-containing medium to Panserin ProVero can be done without any special adaption procedures. For those cells which do not tolerate adaption procedures. For those cells which do not tolerate an immediate switch we recommend a primary culture with Panserin ProVero supplemented with serum and a stepwise reduction of serum towards a serum-free Panserin ProVero cultivation. This stepwise adaption will be supported by higher cell seeds (20,000 cells/cm^2) or by using the reduced serum concentration after an attachment phase of the adherent cells with higher FBS content. For the successful transfer into serum-free cultivation the vitality of the cells is an important factor. Thus the cells should be transferred in the logarithmic growth phase. According to our experience a transfer within the stationary growth phase will show lower prospects of success. For adherent cells it should be assured that - if trypsin is used for detachment - the enzyme is completely washed out or is inactivated by trypsin-inhibitors, because serum is not present for a neutralizing effect.

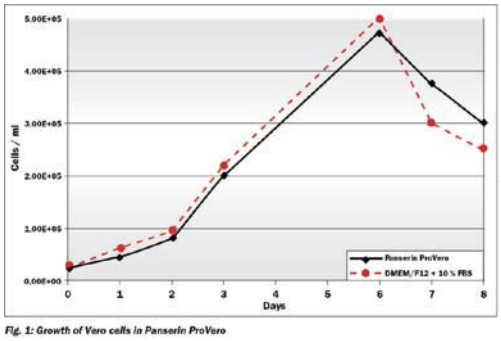


Fig. 1: Growth of Vero cells in Panserin ProVero

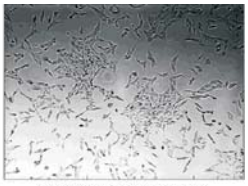


Fig. 2:Vero-cells in Panserin ProVero

PANSERIN ProVero	100 ml	P04-710613M
	500 ml	P04-710613

PANSERIN S2

Panserin S2 is a protein-free medium for an optimized growth of insect Drosophila S2 cells in suspension culture. Insect cells are widely used for the industrial production of recombinant proteins.

Composition:
Panserin S2 contains amino acids, vitamins, salts, trace elements, lipids and growth promoting factors in a formulation optimized for the growth of insect cells. It contains no protein or any further components of human or animal origin.

Suitability:
Panserin S2 is suitable for the cultivation of Drosophila S2 cells and the production of recombinant protein. (e.g. Baculovirus expression vector system, BEVS)

Special Advantages:
Panserin S2 with its protein-free formulation is free of human and animal components. This allows the production of recombinant proteins for medical and therapeutic purposes. The protein-free formulation also facilitates convenient and economic purification of final products from the cell culture. Panserin S2 guarantees a high cell density and viability resulting in an increased production and easy and economic purification of recombinant protein. (Baculovirus expression vector system)

Application:
The optimal temperature range for most insect cells is 25 °C - 30 °C (27 °C incubation ±0.5 °C). The pH for cell culture with Lepidoptera cell cultures should be between pH 6.0 to pH 6.4. The osmolality for insect media should be 345 - 380 mOsm/kg. For optimized oxygen supply, slightly unscrew the caps of the culture vessels.

Adaption to a protein-free culture:
Insect cells from a serum containing culture should be adapted to the protein-free culture. This could be done either by direct or sequential adaptation. Suspension cells should be taken from the middle exponential growth phase with a vitality of over 90% (Trypan Blue Exclusion Staining).

Direct adaptation to PANSERIN S2:

- Transfer the cells from serum containing culture (e.g. Schneider's Drosophila Medium, FBS 5-10%) directly into preheated (27 °C) protein-free medium Panserin S2 with a cell density of 1-2 x 10^6 cells/ml.
- When the culture reaches a cell density of > 4x10^6 cells/ml (after 4-7 days), subculture cells in new protein-free medium with a cell density of 5x10^5-1x10^6 cells/ml.
- Repeat subculture until a vitality of at least 80% is obtained.

Note: S2 cells do not grow well when seeded at a density below 5x10^5-1x10^6 cells/ml.

Indirect adaptation to PANSERIN S2:

- Subcultivate cells derived from serum containing culture in a 1:1 mixture with the original culture medium and Panserin S2. Seeding density 1-2x10^6 cells/ml
- When the culture reaches a cell density of > 4 x 10^6 cells/ml, subculture the cells with fresh serum-free medium in a 1:1 mixture.
- Repeat this process until serum levels are below 0.1% and the cell vitality is > 80%. The cell number should exceed 5 x10^6 cells/ml.
- For general maintenance of cells, pass S2 cells when cell density is between 6 to 20 x 10^6 cells/ ml and split at a 1:2 to 1:5 dilution.

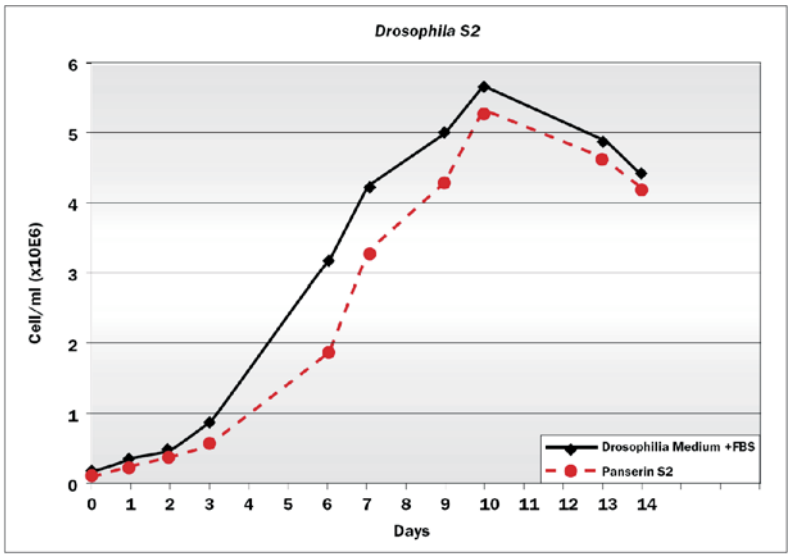


Fig. 2: Growth of Drosophila S2 in Panserin S2

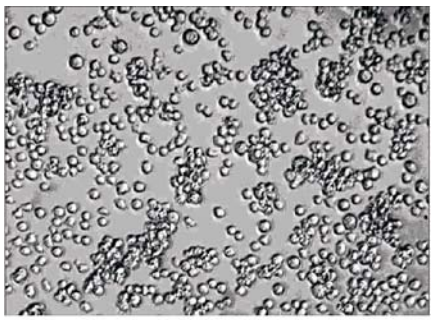


Fig. 1:Drosophila S2-cells in Panserin S2

PANSERIN S2	100 ml 500 ml	P04-710210 P04-710200

SPODOPAN

SPODOPAN is a protein-free medium for an optimized growth of insect cells such as Sf9 and Sf21 (Spodoptera frugiperda) in suspension culture. Insect cells are often used for the industrial production of recombinant proteins.

Storage conditions:
Storage: +2 °C to +8 °C
Stability: 1 year
Filling: 100 ml, 500 ml, other fillings on request

Composition:
SPODOPAN contains amino acids, vitamins, salts, trace elements, lipids and growth promoting factors in a formulation optimized for insect cells. It contains no protein or any orther components of human or animal origin.

Suitability:
SPODOPAN is suitable for the cultivation of insect cells and the production of recombinant proteins. (Baculovirus expression vector system, BEVS)

Special Advantages:
SPODOPAN with its protein-free formulation is free of human and animal components. This allows the production of recombinant proteins for medical and therapeutic purposes. The protein-free formulation also facilitates an easier and more economic purification of final products from the cell culture. SPODOPAN guarantees a high cell density with increased production of recombinant proteins (Baculovirus expression vector system).

Instructions for use:
Adaption to a protein-free culture

The optimal temperature range for most insect cells is 25 °C to 30 °C (27 °C incubation ±0.5 °C).

The pH for cell culture with Lipidopteran cells should be between pH 6.0 and pH 6.4.

The osmolality for insect cells media should be 345 – 380 mOsm/kg.
For optimized oxygen supply, slightly unscrew the caps of the culture vessels.

Insect cells from a serum-containing culture should be adapted to the protein-free culture. This could be done either by direct or sequential adaptation.

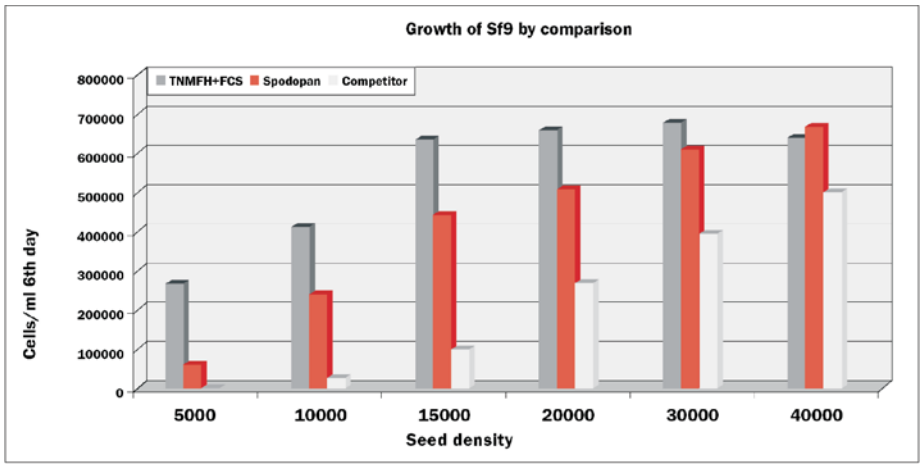
Suspension cells should be taken from the middle exponential growth phase with a vitality of over 90% (Trypan Blue Exclusion Staining).

Direct Adaptation to SPODOPAN:

- Transfer the cells from the serum-containing culture (e.g. TNM-FH, FBS 5-10%) directly into preheated (27 °C) protein-free medium (SPODOPAN) with a cell density of $5x10^5$ cells/ml.
- When the culture reaches a cell density of > $2 x 10^6$ cells/ml (after 4-7 days) subculture cells in new proteinfree medium with a cell density of $5x10^5$ cells/ml.
- Repeat subculture until a vitality of at least 80% is obtained.

Indirect adaptation to SPODOPAN:

- Subcultivate cells from the serum-containing culture in a 50:50 ratio with the original culture medium and SPODOPAN. Seeding density $5x10^5$ cells/ml
- When the culture reaches a cell number of > $1x10^6$ cells/ml subculture the cells with fresh protein-free medium in a 1:1 ratio.
- Repeat this process until serum levels are below 0.1% and the cell vitality is > 80%. The cell number should exceed $1x10^6$ cells/ml.



Sf9 cells in Spodopan

SPODOPAN	100 ml	P04-850100
	500 ml	P04-850500

ENDOPAN 300 SL

ENDOPAN 300 SL is the first complete medium specially developed for the serum-free in vitro culture of human endothelial cells containing all components necessary for optimal growth.

Endothelial cells line blood and lymphatic vessels and the internal cavities of the heart. They display a strongly flattened, polygonal form and mostly rest on a basal membrane. They adhere to each other by desmosomes and tight-junctions. With a total cell number of about one trillion (1012), the endothelium is one of the biggest organs of the body and plays a key role in many physiological and patho-physiological processes (e.g. cell-based immune response, wound healing, inflammation, allergy, cardiovascular diseases, tumour growth). A huge number of soluble factors circulating in the blood or released by neighbouring cells control proliferation or apoptosis of endothelial cells and the invasion and migration of leucocytes to the endothelium, thereby regulating the maintenance, degeneration, or regeneration of blood vessels.

Composition and Application:
ENDOPAN 300 SL ready-to-use is a complete medium specially developed for serum-free in vitro culture of human endothelial cells and it contains all components necessary for optimal growth. It is designed for use in an incubator at 37 °C with a 5% CO_2 atmosphere. ENDOPAN 300 SL kit is provided with a serum substitute (PANEXIN SL-S) and supplements in separate sterile packings.

ENDOPAN 300 SL has been designed for serum-free culture of endothelial cells directly after isolation. This exclusive medium is optimized for the maintenance and expansion of endothelial cells under serum-free culture conditions. HUVEC cultured in ENDOPAN 300 SL exhibit a typical endothelial morphology and express endothelial specific markers such as CD31 or von Willebrand Factor and bind UEA-1 lectin. Additionally, HUVEC in ENDOPAN 300 SL have been shown to maintain endothelial cell signal transduction pathways. When using complete ENDOPAN 300 SL the growth rate of HUVEC is similar to that obtained for cells cultured in endothelial growth media containing bovine serum and supplements.

Suitability:
ENDOPAN 300 SL is suitable for the culture of:
Human Umbilical Vein Endothelial Cells
Human Umbilical Artery Endothelial Cells
Human Pulmonary Artery Endothelial Cells
Human Saphenous Vein Endothelial Cells

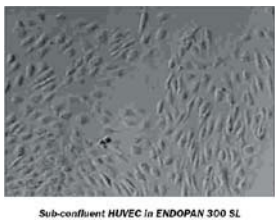
Although not extensively tested, it has been shown that ENDOPAN 300 SL can also be used with endothelial cells of bovine, pig, rat, and rabbit origin.

Special Advantages:
Endothelial cell biology has been greatly advanced by studying cultured vascular endothelial cells in vitro. Traditionally, complete endothelial growth media contain animal serum. The advance of so-called low-serum media for endothelial cells has improved the quality of experimental data acquired in recent years. However, endothelial cells may synthesize substances which can not be detected due to their low quantity or masking effects from serum.

In the past, cellular signalling pathways in endothelial cells have not been decipherable experimentally because even low concentrations of serum present in traditional media induce an undefined and undesired stimulation of cell surface receptors or intracellular signalling which only may become evident under serum-free conditions. As endothelial cells move into the field of interest for vascular tissue engineering with potential therapeutic application, the presence of whole animal serum is undesirable for such applications.

Instructions for use:
It is recommended to use serum-free conditions from the start. However, sequential adaptation or adaptation with conditioned media over several passages which is usually necessary when switching from serum-supplemented to serum-free culture conditions is not necessary with this medium. It is not recommended to switch to ENDOPAN 300 SL directly after splitting the cells. Please allow adhesion of trypsinized cells for 24h before changing the medium to serum-free.

ENDOPAN 300 SL may also be used on previously serum exposed endothelial cells with very little weaning. Some cells may not survive a direct exposure to serum-free conditions and the cell population may show some delay in performance or slight changes in morphology. Minor changes in cellular appearance should not be a matter of concern as long as viability and proliferation remain at usual level. A high cell density (close to confluence) before the adaptation increases survival rates and sequential seeding at higher density will help cells to perform well in the new culture environment.



Sub-confluent HUVEC in ENDOPAN 300 SL

Confluent HUVEC in ENDOPAN 300 SL

Endopan 300 SL ready-to-use	500 ml	P04-00650
Endopan 300 SK kit with supplements	500 ml	P04-0065K

Reagents

PANEXIN SL-S Serum Substitute for HUVEC cultures	25 ml	P04-90065S
SL-S Trypsin/EDTA	50 ml	P10-0231SF
SL-S Trypsin-Inhibitor	50 ml	P10-0331SF
SL-S Medium (Working Medium)	500 ml	P04-300500
SL-S Collagen 0.01 %	25 ml	P06-20650
SL-S Cryopan	25 ml	P07-94050

PANSERIN 411

Panserin 411 is a complete ready to use medium for the serum-free cultivation of a multitude of adherent and nonadherent cells which are Insulin-dependent (e.g. CHO-cells).

Storage conditions:
Storage: +2 °C to +8 °C
Stability: 8 months
Filling: 100 ml, 500 ml, other fillings on request

Composition:
Based on Iscove's MEM, trace elements, albumin, cholesterol, soya lipids, vitamins and insulin were added to the medium. It does not contain any growth or attachment factors.

Suitability:
Panserin 411 is a multi-purpose medium suitable for a variety of cells. In Panserin 411 adherent as well as nonadherent cells can be cultivated. As the medium contains no growth factors there is a possibility to investigate the effects of specific growth factors added to the cell culture. Panserin 411 does not contain any attachment factors. With some cell types a pre-treatment of the cell culture vessels with gelatine, collagen, poly-D-lysine or fibronectin may support or enable a culture under serum-free conditions. Please note that a coating may be especially important with low seeding densities. With every adaption to serum-free media, changes of the cells should be taken into consideration. These changes may concern morphology, karyotype, surface markers and so on. Thus cells in serum-free medium may not be identical with those from cultures containing serum in which they originated (selection).

Instructions for use:
In many cases the switch from serum-containing to serum-free cultivation can be done without any special adaption procedures. For those cells which do not tolerate an immediate switch we recommend a primary culture with serum containing medium and a stepwise reduction of medium towards a serum-free cultivation. We can provide you with an adaption protocol for many different cell types. This stepwise adaption will also be supported by higher cell seeds or using a lowered serum concentration after attachment of the cells in medium containing a higher amount of serum. For the successful transfer into serum-free cultivation the vitality of the cells is an important factor. Thus the cells should be transferred in the logarithmic growth phase. According to our experience the transfer in the logarithmic growth phase will have higher prospects of success. In adherent cells it should be assured that - if trypsin is used for detachment - the enzyme is completely washed out or is inactivated by trypsin-inhibitors because there is no trypsin inactivating effect of FBS; use trypsin-inhibitor to stop trypsin activity. In some cases of very sensitive cells it could be also reasonable to do the stepwise adaption and dilution not only with serum but also in the medium which has been used so far.
Studies on the effect of externally added growth factors will be more valid. For cells which are dependent on specific growth factors these factors should be added in the required concentrations.

PANSERIN 411	100 ml 500 ml	P04-710411M P04-710411

PANSERIN 411S

Panserin 411S is a complete ready to use medium for the serum-free cultivation of myeloid and lymphoid cells for cytological examination.

Composition:
Based on RPMI 1640 medium, additional trace elements, albumin, cholesterol, soy lipids, vitamins and hormones are added.

Suitability:
Panserin 411S is a serum-free complete medium for the cultivation of myeloid and lymphoid cells from peripheral blood or bone marrow. It is therefore suitable for a rapid expansion of blood cells in order to investigate leukemic diseases (ALL, AML, CLL, CML, MPN, MDS). The state of the art diagnostic techniques of leukemic diseases are based on the interaction of cytomorphology including cytochemistry with immunophenotyping, chromosome banding analysis, FISH and molecular genetics. In Panserin 411S the number and quality of metaphases are significantly higher and independent of individual batches as compared to serum-containing media.

Instructions for use:
Cells ($1x10^7$) are seeded in 5 ml Panserin 411S. Depending on the assay or quality of raw material, an unstimulated culture and another 1-3 cultures with appropriate growth factors are prepared. The culture time is 24 to 72 hours at 37 °C in an incubator with 5% CO2 gasing.

The processing of the metaphases is done with hypotonic KCl solution and Carnoy´s fixative.

PANSERIN 411S	500 ml 1000 ml	P04-7411S1 P04-71411S

PANSERIN 412

Panserin 412 is a complete ready for use medium for the serumfree cultivation of a multitude of adherent cells.

Composition:
Based on Iscove's MEM, trace elements, albumin, cholesterol, soya lipids, vitamins and insulin were added to the medium. It does not contain any growth or attachment factors.

Suitability:
Panserin 412 is a multi-purpose medium suitable for a variety of adherent cells. Panserin 412 contains special attachment factors for the successful cultivation of cells that normally only hardly attach. As the medium contains no growth it is possible to investigate the special effects of added growth factors to the cell culture. With every adaption to serumfree media, changes of the cells should be taken into consideration. These changes can concern the morphology, the karyotype, the surface marker etc. Thus cells in serumfree medium don't always have to be identical with those from the culture containing serum in which they originate (selection).

Application:
In many cases the switch from serum-containing to serumfree cultivation can be done without any special adaption procedures.
For those cells which do not tolerate an immediate switch we recommend a primary culture with serum containing medium and a stepwise reduction of medium towards a serumfree cultivation. We can provide you with an adaption protocol for many cells.
This stepwise adaption will also be supported by higher cell seeds or by using the lowered serum concentration after attachment in adherent cells. For the successful transfer into a serumfree cultivation the vitality of the cells is an important factor. Thus the cells should be transferred in the logarithmic growth phase.
According to our experience the transfer within the stationary growth phase will have lower prospects of success. In adherent cells it should be assured that – if trypsin is used for detachment – the enzyme is completely washed out or is inactivated by trypsin-inhibitors in order for the serum to have no neutralizing effect.
In some cases of very sensitive cells it could be also reasonable to do the stepwise adaption and dilution not only with serum but also with the used medium.
Panserin media were developed to support the cell growth without the use of serum. Thus the Panserin 412 does not contain any further growth factors. The analysis of externally added growth factors will be more specific. For cells which are dependent on specific growth factors these factors should be added in the required concentrations.

PANSERIN 412	100 ml 500 ml	P04-710412M P04-710412

1

PANSERIN 413

Panserin 413 is a ready to use medium for the cultivation of lymphocytes from whole blood.

Storage conditions:
Storage: +2 °C to +8 °C
Stability: basic medium: 1 year, supplements: 2 years
Filling: 100 ml, 500 ml, other fillings on request

Composition:
Based on RPMI 1640/DMEM-F12, trace elements, albumin, cholesterol, soya-lipids and vitamins were added to the medium. A growth factor mixture is also supplied which has to be added to the medium immediately before use.

Suitability:
Panserin 413 has been developed for the cultivation of lymphocytes from whole blood. Normally blood cells die rather quickly in culture, only lymphocytes can be cultivated over multiple divisions in culture. To achieve a division of non-proliferating cells, the cells must be stimulated with certain mitogens. These mitogens are mostly herbal lectins (phytohemagglutinin, PHA).

Instructions for use:

1. **Isolation of lymphocytes from whole blood using density gradient centrifugation.**

- Mix heparinised blood 1 : 1 with DPBS and add it into a centrifuge tube which has been filled with lymphocyte separating medium (Pancoll density 1,077 g/ml): pipette carefully in order to avoid a phase mixture!
- Centrifuge the gradient at 400 x g for 30 minutes at ambient temperature (brake of the centrifuge set on "off"); 4 phases will be visible:
  - top phase plasma
  - opaque whitish bands (lymphocytes)
  - separating medium
  - pellet with erythrocytes and granulocytes
- Aspirate the plasma with a pipette and transfer the lymphocytes with a fresh pipette into a new centrifuge tube.
- Wash the lymphocytes with DPBS (without Ca, Mg) and centrifuge them at 100 x g for 10 minutes. Repeat washing step.

2. **Cultivation and stimulation of the lymphocytes.**

- Resuspend lymphocytes in Panserin 413.
- For the stimulation of the lymphocyte proliferation adjust the cells to a cell density of approx.1 x 10^5 and add phytohemagglutinin at a concentration of 2 till 10 µg/ml; the incubation time is 48 to 72 hours, depending on the kind and origin of the lymphocytes and depending on further use.
- Cultivation in Panserin 413. After about 14 days cells have to be restimulated.

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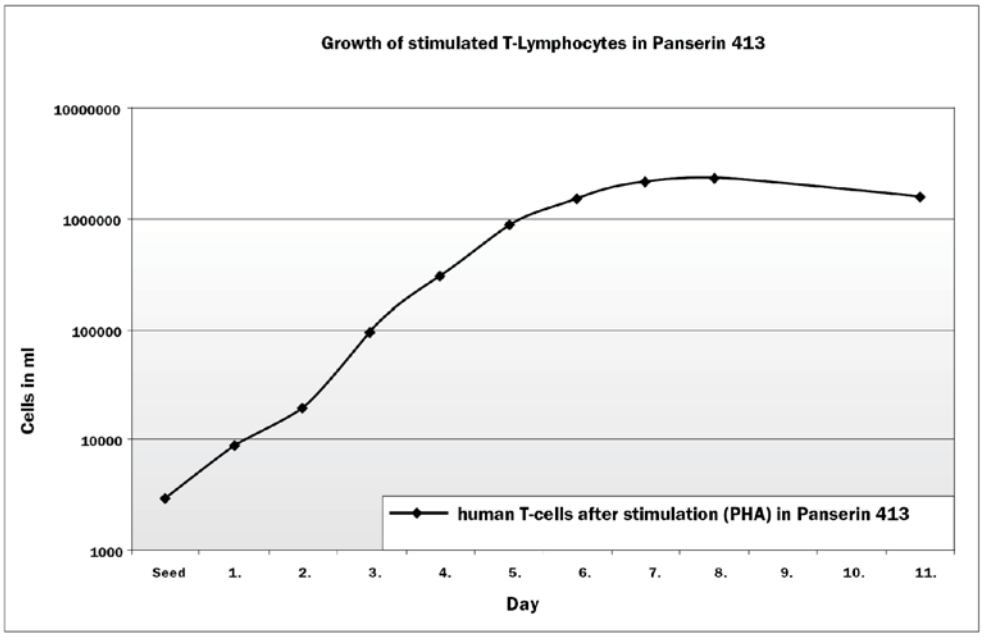
PANSERIN 413



PANSERIN 413 with one supplement	500 ml	P04-710413

PANSERIN 416

Panserin 416 is a serumfree medium (basic medium) which is, after supplementation of growth factors, suitable for the production of dendritic cells.

Composition:
Based on RPMI 1640/DMEM/F-12, trace elements, albumin, cholesterol, soya-lipids and vitamins were added to the medium. A growth factor mixture is also supplied which has to be added to the medium shortly before cultivation.

Suitability:
Dendritic cells are highly specialized antigen-presenting cells and can initiate and regulate antigen-specific immunoresponses. This ability can be used in order to generate immunoresponses against certain proteins of tumour cells and thus the immune system itself could be able to fight against tumours. Dendritic cells have been isolated from a great variety of non-lymphatic and lymphatic tissue of human beings, mice and other species.

For the generation of tumour vaccines, dendritic cells can be produced from the peripheral blood of tumour patients. In clinical studies the principal effectiveness of a vaccination with dendritic cells has been shown.

Antigen Uptake:
In almost any tissue of the body, dendritic cells form a dense network of guardian cells that take up extracellular components with processes such as phagocytosis and endocytosis and thus analyse their environment. Proteins that have been taken up undergo an intracellular decomposition into peptides, are bound to MHC-molecules and transported to the cell surface. Thus antigenic determinants of the peptides are made recognizable to T-cells. Within the scope of the physiological cell regeneration dendritic cells leave the peripheral tissue and migrate with the lymph into a regional lymph node where they interact with T-cells. From an intact tissue dendritic cells reach the lymph node in an inactivated condition.

A functioning monitoring system excels in the ability to quickly and specifically recognize damaging processes and to take suitable steps against them. For this purpose, dendritic cells carry receptors on their surface for a variety of danger signals which can be radiated from micro-organisms, mediators inherent in the body or activated T-cells. Examples for microbial structures activating dendritic cells are lipopolysaccharides of gram-negative bacteria, cytidine guanosine dinucleotide (cpG) – rich bacterial DNA and viral double stranded RNA. Endogenous mediators, for which dendritic cells have special receptors and which send an activating signal, are cytokines, prostanoids and adenine nucleotides. Activated T-cells can stimulate dendritic cells by means of the CD40-ligand integrated in their cell membrane. The activation of these different receptors induces essential cell changes which are summarized by the term maturation. The ability of phagocytosis gets lost.

Peptides bound to MHC-molecules are presented in a higher density and with greater stability. The cytoskeleton structure is reorganized and a changed expression of chemokine receptors enables the dendritic cells to get from the focus of inflammation into the draining lymph

PANSERIN 416

node. Co-stimulating molecules on the surface of dendritic cells and the release of cytokines, e. g. interleukin-12, finally allow the dendritic cells an efficient interaction with T-cells.

Induction of an Immunoresponse:
In the lymph node, dendritic cells interact with various lymphocyte populations. Above all T-cells, which haven't yet had any antigen contact, feel the cell surface of dendritic cells and are activated if the T-cell receptor recognizes the presented antigen. This central process for the acquired (antigen-specific) immunoresponse is called "Priming". Cytotoxic T-cells develop from CD8 cells and are able to kill those cells which are recognized by their T-cell receptor.

Tumour Vaccination with Dendritic Cells:
Tumour cells express specific proteins which can be recognized as antigenic determinants by T-cells. As a rule, however, this is not sufficient for the immune system to generate an effective immunoresponse against tumour cells; there is rather a tolerance. On one hand this is because tumourassociated antigens are often also found in sound tissue in a low density; on the other hand tumour cells have numerous strategies to escape an immunoresponse. In a number of animal experiments, however, it could be clearly shown that this tolerance for tumours can be broken by a vaccination with dendritic cells.

Generation of Dendritic Cells:
Dendritic cells are derived from hematopoietic precursor cells in the bone marrow. Three different subpopulations with each characteristic features and functions are described for the human being: myeloid dendritic cells, plasmacytoid and Langerhans cells of the skin. For tumour vaccinations, myeloid dendritic cells are mainly of interest as they are especially capable of taking up and presenting antigens. Dendritic cells with myeloid characteristics can be produced by an in vitro culture of monocytes in the presence of the cytokines interleukin- 4 (IL-4) and granulocytes- macrophages colony-stimulating factor (GM-CSF). Alternatively dendritic cells can be generated from CD34+ hematopoietic stem cells of the peripheral blood. By the addition of growth factors, e.g. Klt3-ligand, dendritic cells in the blood, which normally make up only approx. 0,1 - 0,5 % of the mononuclear cells, can be expanded many times over.

After activation, dendritic cells reach their full capacity for the T-cell stimulation. For the maturation of the dendritic cells, cytokines (TNF alpha), LPS or monocyte conditioned medium are used.

Production and serumfree cultivation of dendritic cells from mononuclear cells of peripheral blood (PBMC).

Instructions for use:
Separation of Blood with Separating Medium (Pancoll 1,077 g/ml)

The blood samples should be processed as soon as possible after production in order to achieve optimal results. A storage of the blood samples for 24 hours at ambient temperature causes among other things a reduced output of lymphocytes, a change of the surface markers and a reduced response on mitogen stimulation.

1) Add Pancoll (3 ml) into a suitable, sterile centrifuge tube under sterile conditions.
2) Carefully coat the separating medium with the diluted blood sample (4 ml). Important: Don't mix the blood sample with Pancoll!
3) Centrifugation at 400 x g for 30 - 40 minutes at 18 - 20 °C.
4) After the centrifugation, carefully take off the upper phase (containing serum and platelets) with a pipette without mixing the interphase with the lymphocytes.
5) Transfer the lymphocyte band into a new centrifuge tube with a new pipette. Here it is important to take off the whole material of the interphase with as little volume as possible.
6) Add at least 3 volumes of a physiological saline solution (6 ml) to the lymphocytes.
7) Carefully suspend the lymphocytes with a pipette.
8) Centrifugation at 60 - 100 x g for 10 minutes at 18 - 20 °C.
9) Reject the supernatant. Repeat washing step (point 6 - 9).

Serumfree Cultivation of Dentritic Cells in Panserin 416:
After the last washing step the mononuclear cells are transferred with a cell density of 1 x 10^7 cells/ml into Panserin 416. In order to remove non-adherent cells, the culture dishes are put into the incubator for 2 hours. Then the supernatant is carefully taken off and replaced by new Panserin 416. GM-CSF (800 U/ml) and interleukin-4 (500 U/ml) are added as growth factors. The culture dishes are incubated for another 6 days in the incubator and every day half of the medium is replaced by new medium which is supplemented by GMCSF and IL-4.

PANSERIN 416 with one supplement	500 ml	P04-710416

PANSERIN 604ST

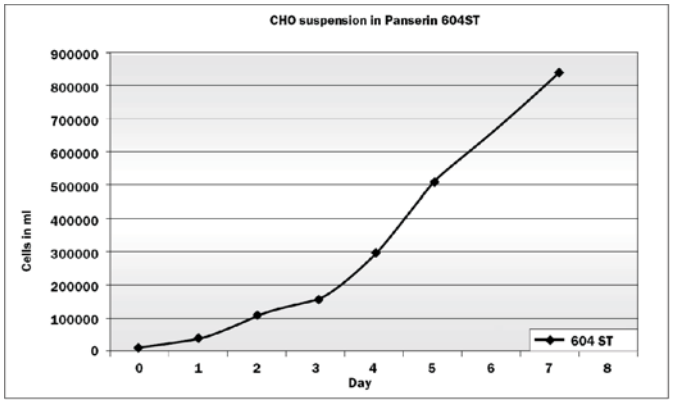
Panserin 604ST is a ready to use complete medium for the cultivation of transfected and non-transfected CHO cells (Chinese Hamster Ovary) in suspension culture.

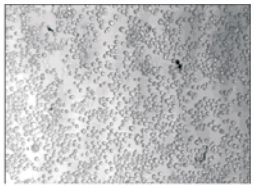
Composition:
Panserin 604ST is a completely defined medium for the cultivation of CHO cells in suspension culture. It contains no human or animal ingredients. It is complemented by recombinant proteins in low concentration. This allows a simple purification of the final products.

Suitability:
Cultivation of transfected and non-transfected CHO cells in suspension culture (bioreactor) for the production of recombinant proteins.

Special Advantages:
Panserin 604ST contains no undefined lysates or herbal hydrolysates. Panserin 604ST allows short adaptation phases and very good growth rates. Through an optimal and balanced composition of the medium an aggregate formation of the cells, as observed in many serumfree media, could be largely avoided. Particularly suitable for the production of recombinant proteins combined with a easier purification of the final products due to the low protein content of the medium.

Instructions for use:
In many cases the switch from serum-containing medium to Panserin 604ST can be done without any special adaption procedures. For those cells which do not tolerate an immediate switch we recommend a primary culture with Panserin 604ST supplemented with serum and than a stepwise reduction of serum towards a pure Panserin 604ST cultivation.
This stepwise adaption will also be supported by higher cell seeds. Cells should be seeded in a density of at least 5 x 10^4 cells/ml.
After a few passages in serumfree culture at lower growth rates the cells reach high growth rates. For a successful transfer into serumfree cultivation the vitality of the cells is an important factor. Thus the cells should be transferred in the logarithmic growth phase. According to our experience a transfer within the stationary growth phase will have lower prospects of success.
In adherent cells it should be assured that – if trypsin is used for detachment – the enzyme is completely washed out or is inactivated by trypsin-inhibitors in order for the serum to have no neutralizing effect.





CHO cells in Panserin 604ST

PANSERIN 604ST	500 ml	P04-604ST

PANSERIN 604SPF

Panserin 604SPF is a ready to use complete medium for the cultivation of transfected and non-transfected CHO cells (Chinese Hamster Ovary) in suspension culture.

Composition:
Panserin 604SPF is a completely defined medium for the cultivation of CHO cells in suspension culture. It contains no protein and also no human or animal ingredients. This allows a simple purification of the final products and the use in critical applications such as drug production.

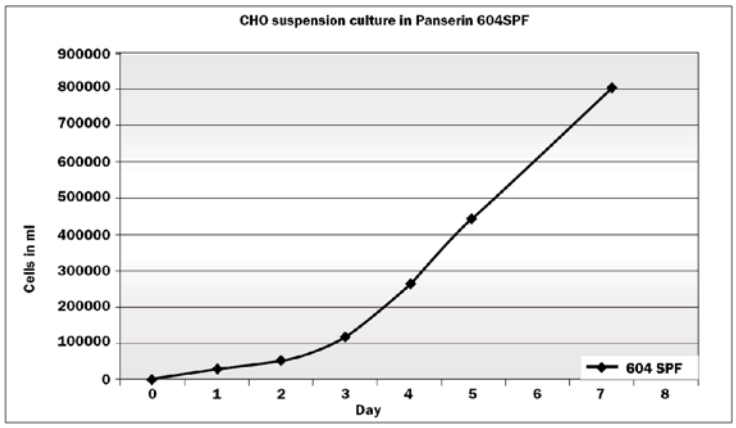
Suitability:
Cultivation of transfected and non-transfected CHO cells in suspension culture (bioreactor) for the production of recombinant proteins.

Special Advantages:
Panserin 604SPF contains no undefined lysates or herbal hydrolysates. Panserin 604SPF allows short adaptation phases and very good growth rates. Through an optimal and balanced composition of the medium an aggregate formation of the cells, as observed in many serumfree media, could be largely avoided. Particularly suitable for the production of recombinant proteins combined with a easier purification of the final products due to the low protein content of the medium.

Instructions for use:
In many cases the switch from serum-containing medium to Panserin 604SPF can be done without any special adaption procedures. For those cells which do not tolerate an immediate switch we recommend a primary culture with Panserin 604SPF supplemented with serum and than a stepwise reduction of serum towards a pure Panserin 604SPF cultivation. This stepwise adaption will also be supported by higher cell seeds. Cells should be seeded in a density of at least 5x 10^4 cells/ml. After a few passages

PANSERIN 604SPF

in serumfree culture at lower growth rates the cells reach high growth rates. For the successful transfer into serumfree cultivation the vitality of the cells is an important factor. Thus the cells should be transferred in the logarithmic growth phase. According to our experience a transfer within the stationary growth phase will have lower prospects of success. In adherent cells it should be assured that – if trypsin is used for detachment – the enzyme is completely washed out or is inactivated by trypsin-inhibitors in order for the serum to have no neutralizing effect.



PANSERIN 604SPF	500 ml	P04-604SPF

PANSERIN 701

Panserin 701 is a complete ready to use serum-free medium for the cultivation of lymphocytes from whole blood.

Storage conditions:
Storage: -20 °C
Stability: 1 year
Filling: 100 ml, 500 ml, other fillings on request

Composition:
Based on Iscove's MEM the medium is enriched with additional trace elements, albumin, cholesterol, lipids and vitamins. It contains the mitogen phythemagglutinin (PHA) for a growth stimulation of lymphocytes.

Suitability:
Panserin 701 has been developed for the serum-free cultivation of lymphocytes from whole blood. The herbal lectin (PHA) in Panserin 701 stimulates cell division.

Instructions for use:

1. Isolation of lymphocytes from whole blood using density gradient centrifugation.

- Mix heparinzed blood 1 : 1 with DPBS and place centrifuge tubes, which were previously filled with lymphocyte separation medium (Pancoll, density 1.077 g / ml), pipette carefully to avoid mixture of the different phases.
- Centrifuge the gradient at room temperature for 30 minutes at 400 x g (brake of the centrifuge should be set at off); centrifugation will reveal 4 phases:
  - Top layer plasma
  - Opaque white layer (lymphocytes)
  - Separation medium
  - Pellet with erythrocytes and granulocytes
- Remove the plasma with a pipette and transfer the lymphocytes with a fresh pipette into a new centrifuge tube.
- Wash the lymphocytes with DPBS (without Ca, Mg) and centrifuge at 100 x g for 10 minutes. Repeat washing procedure.

2. Cultivation and stimulation of lymphocytes.

- Resuspend lymphocytes in Panserin 701 at a cell density of 1 x 10^5. The phytohemagglutinin (PHA) in Panserin 701 stimulates the proliferation of lymphocytes.
- Incubate for 48 to 72 hours, depending on the nature and origin of lymphocytes and on future use.
- Cultivation can be done with Panserin 413. Restimulation with Panserin 701 after 14 days may be necessary.

PANSERIN 701	100 ml 500 ml	P04-710701M P04-710701

PANSERIN 801

Panserin 801 is a serumfree ready to use medium for the cultivation of human keratinocytes.

Composition:
MCDB-153 is used as the basal medium which must be supplemented with the supplied supplements just before the use. These supplements are:

- Epidermal Growth Factor (EGF)
- Insulin
- Hydrocortisone
- Ethanolamine
- Phosphoethanolamine
- Pituitary Extract (BPE)

Suitability:
Panserin 801 has been developed for the serumfree cultivation of human keratinocytes. Panserin 801 selectively supports the growth of human keratinocytes and concurrently prevents the overgrowth with fibroblasts.

Application:

- Thaw the supplied supplements.
- Add the basal medium under sterile conditions.
- Trypsinate keratinocytes, then wash the cells and stop the remaining trypsin activity with a trypsin inhibitor (10 mg/ml).
- Wash the cells again and determined cell amount.
- Adjust the cell density at 3.000 – 5.000 cell/ml in the following subcultivation (initial seeding density 1 - 5 x 10^6 cells/cm^2). Incubate keratinocytes with supplemented Panserin 801 in an incubator.
- Cell culture dishes could be precoated alternatively with attachment factors (collagen, fibronectin or natural/ synthetic fragments of these factors).

PANSERIN 801 with 6 supplements	500 ml	P04-710801

PANSERIN PX10

Panserin PX10 is a ready to use serum-free complete medium for the cultivation of myeloma- and hybridomacells for the production of monoclonal antibodies.

Composition:
Based on RPMI 1640/DMEM/F-12, trace elements, albumin, cholesterol, soya-lipids, vitamins and hormones were added to the medium. The medium does not contain any growth hormones.

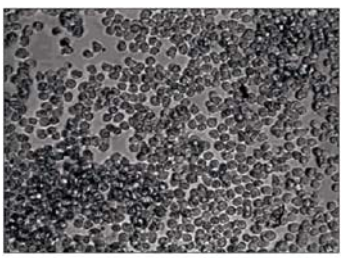
Suitability:
Cultivation of myeloma- and hybridoma-cells for the production of monoclonal antibodies.

Special Advantages:
Panserin PX10 is a ready to use serum-free medium for the production of monoclonal antibodies. It contains no undefined lysates or peptones hydrolysates. Due to its optimized composition Panserin PX10 shows significant growth stimulation even at low seeding densities. In addition to the excellent growth properties Panserin PX10 shows excellent cloning properties.
Conventional Serum-free Systems often require long and laborious steps and seeding densities of up to 10^5 cells/ml. In contrast, most clones could be directly transferred into Panserin PX10 culture. With Panserin PX10 clones can be built without major difficulties.

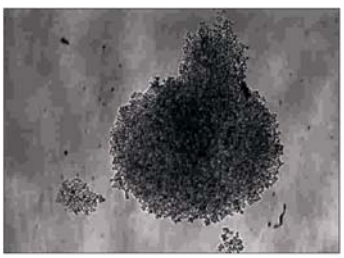
Instructions for use:

- Warm up Panserin PX10 to 37 °C.
- Transfer hybridoma directly into Panserin PX10. In most cases a cell number of 1000 cells/ml is sufficient.
- Incubate the cells in the usual way in the CO_2-incubator at 37 °C (5 % CO_2-fumigation) res. multiply them in the bioreactor.
- Extract monoclonal antibodies from the supernatant.

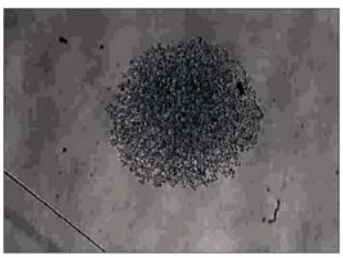
In many cases the transfer from serum-containing medium to Panserin PX10 can be done without any special adaption procedures. For those cells which do not tolerate an immediate switch we recommend a primary culture with Panserin PX10 supplemented with serum and than a stepwise reduction of serum towards a pure Panserin PX10 cultivation. Although Panserin PX10 supports the growth of even low cell densities, during the transfer to the serumfree culture the cells should be seeded in higher densities (5 x 10^3 to 5 x 10^4 cells/ml). For the successful transfer into a serumfree cultivation the vitality of the cells is an important factor. Thus the cells should be transferred in the logarithmic growth phase. According to our experience the transfer within the stationary growth phase will have lower prospects of success.



SP2/0-Ag-14 in Panserin PX10



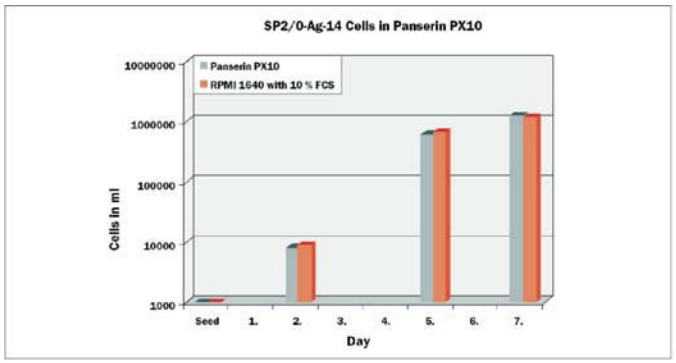
Cloning of SP2/0-Ag-14 in RPMI 1640 with 20% FCS

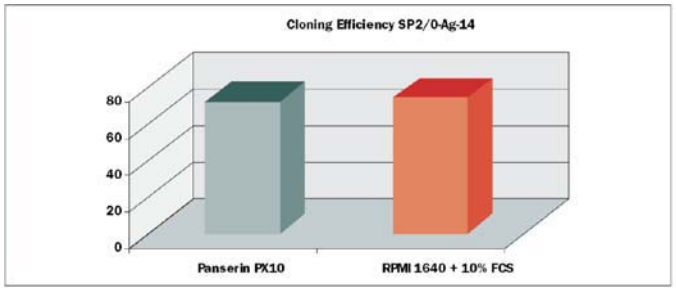


Cloning of SP2/0-Ag-14 in Panserin PX10

PAN™ BIOTECH GmbH

PANSERIN PX10





Typical Growth Curve of SP2/0-Ag-14 in PANSERIN PX10. Sp2/0-Ag-14 cells were transferred from serum containing culture (RPMI 1640 with 10 % FCS) directly into Panserin PX10. Seeding density 1.000 Cells/ml. In comparison Sp2/0-Ag-14 in RPMI 1640 with 10 % FCS.

PANSERIN PX10	500 ml	P04-710PX10

PANSERIN PX40

Panserin PX40 is a ready to use complete medium for the serumfree cultivation of a variety of cells.

Composition:
Based on RPMI 1640/DMEM/F-12, trace elements, albumin, lipoproteins, vitamins, hormones and attachment factors were added to the medium. The medium does not contain any growth hormones.

Suitability:
Cultivation of a variety of adherent cells in a serumfree culture (e. g. HEK, L929, CHO, MDCK, MDBK, 3T3A).

Special Advantages:
Panserin PX40 is a ready to use serumfree medium for the cultivation of a variety of adherent cells. The addition of attachment factors allows the cultivation of even highly demanding cells after a short adaptation phase. It contains no undefined peptones or hydrolysates.

Instructions for use:
Adaptation to the serumfree culture.

Many cell lines can be directly transferred from the serum containing adherent culture in the serumfree culture with Panserin PX40. After a few passages with slower growth afterwards the cells reach growth rates comparable to serum containing culture conditions.

Direct adaptation to Panserin PX40:

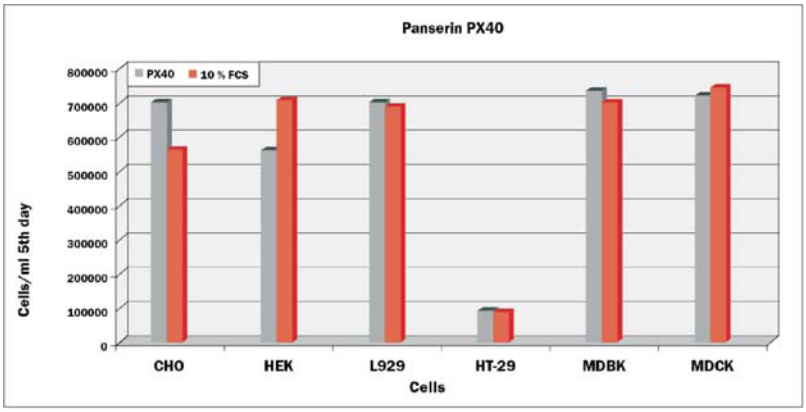
- Use adherent cells in the log phase of a serum containing culture (for example high glucose DMEM with 10 % FCS).
- Evacuate serum containing medium with pipette.
- Wash cell layer with PBS without Ca, Mg.
- Cover cell layer with trypsin / EDTA (0.25 %, 0.02 %) (about 2 ml per T25 bottle).
- Evacuate trypsin after about 1 minute.
- ncubate the cells until the show a round figure and detach from the surface (after about 5 minutes).
- Eliminate remaining trypsin activity with a trypsin inhibitor (1 mg/ml-solution, 1-2 ml trypsin inhibitor solution per T25 bottle).
- Transfer cells into Panserin PX40 and centrifuge again.
- Transfer cells into Panserin PX40 and count the cell number.
- Seed 5 x 10^4 - 1 x 10^5 cells/ml in preheated Panserin PX40. Incubation at 37 °C and 5% CO_2 fumigation in the incubator.
- Transfer cells in fresh Panserin PX40 at a confluence of about 80 %.

Indirect Adaption to Panserin PX40:

- Use adherent cells in the log phase of a serum containing culture (for example high glucose DMEM with 10 % FCS).
- Evacuate serum containing medium with pipette.
- Wash cell layer with PBS without Ca, Mg.
- Cover cell layer with trypsin / EDTA (0.25 %, 0.02 %) (about 2 ml per T25 bottle).

PANSERIN PX40

- Evacuate trypsin after about 1 minute.
- Incubate the cells until the show a round figure and detach from the surface (after about 5 minutes).
- Eliminate remaining trypsin activity with a trypsin inhibitor (1 mg/ml-solution, 1 - 2 ml trypsin inhibitor solution per T25 bottle).
- Transfer cells into Panserin PX40 and centrifuge again.
- Transfer cells into Panserin PX40 and count the cell number.
- Seed 5 x 10^4 - 1 x 10^5 cells/ml in preheated Panserin PX40 with addition of 5 % FCS.
- Incubation at 37 °C and 5 % CO_2 fumigation in the incubator.
- Transfer cells in fresh Panserin PX40 with 2 % FCS at a confluence of about 80 %.
- At the next splitting steps transfer cells in to Panserin PX40 with 1 % FCS and finally with 0,1 % FCS (the same procedure as described).



PANSERIN PX40	500 ml	P04-710PX40

PowerStem ESPro1

PowerStem ESPro1 is an easy to use serum-free medium for cultivation of embryonic stem cells of mice (mES-cells). These pluripotent cells are derived from blastocysts and they can be established to a permanent cell culture. After injection into blastocysts in chimeras, they can form all tissues, including germ cells. In PowerStem ESPro1, the mES-cells largely maintain their undifferented state and can be integrated into the germ line.

Content:
PowerStem ESPro1 medium consists of:

- PowerStem ESPro1 basal medium
- PowerStem ESPro1 growth supplement, which is added at the time of use.
- PowerStem ESPro1 LIF supplement, which is added at the time of use.

Storage conditions:

- PowerStem ESPro1 basal medium: store in the dark at +2 to +8 °C
- PowerStem ESPro1 growth supplement: store in the dark at -20 °C
- PowerStem ESPro1 LIF supplement: store in the dark at -20 °C

Composition:
PowerStem ESPro1 contains purified proteins, lipids, salts, amino acids, trace elements, attachment factors, hormones and growth factors in an optimized formulation. PowerStem ESPro1 is fully chemically defined and contains no peptones or hydrolysates.

Please note: Supplemented PowerStem ESPro1 contains LIF in a concentration of 10 µg/L. If higher levels of LIF are required for your experimental setting, please add additional LIF to the medium.

Suitability:
Serum-free cultivation of embryonic stem cells of mice (mEScells), while maintaining the undifferentiated state. PowerStem ESPro1 is especially designed for the serum-free generation of knockout-mice from genetically modified mES-cells. PowerStem ESPro1 has also been proven to support the serum-free cultivation and expansion of tumor progenitor cells.

Please note: For research use only, not for therapeutic or diagnostic use.

Special advantages:
PowerStem ESPro1 allows the cultivation and expansion of mouse embryonic stem cells (mES-cells) under serum-free conditions. It is fully defined in its composition and thus enables constant and comparable experimental conditions resulting in highly reproducible data. The mES-cell culture can be established without the usual feeder layer (primary fibroblasts), cells show a high proliferation rate and largely retain an undifferentiated state. By adding specific differentiation factors, mES-cells can differentiate in vitro to the desired cell types (e.g. nerve cells, muscle cells, endothelial cells, etc.). Following injection into blastocysts, they can form all tissues in chimeras. Therefore it is possible to generate animals whose genome has been manipulated previously in a cell culture (e.g. knock-out / knock-in mice).

PowerStem ESPro1

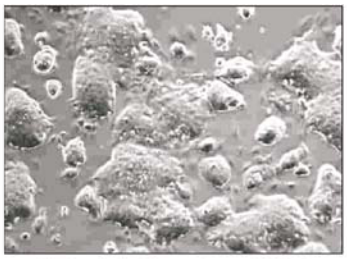
Preparation of PowerStem ESPro1 medium:
PowerStem ESPro1 basal medium requires supplementation with PowerStem ESPro1 growth supplement and PowerStem ESPro1 LIF supplement. To obtain 500 ml PowerStem ESPro1 complete medium please add 50 ml of thawed PowerStem ESPro1 growth supplement and 1ml PowerStem ESPro1 LIF supplement to 450 ml of PowerStem ESPro1 basal medium.

Instructions for use:

- Prepare gelatine-coated plates by covering them with a 0.2% gelatine solution for at least 10 min in the incubator.
- mES-cells should always be kept at a relatively high cell density to maintain their pluripotency.
- The subculture is best carried out from a sub-confluent culture (70% - 80% confluence).
- Individual colonies should not touch each other.
- Too dense growth promotes the differentiation of cells and thus may cause the loss of pluripotency.
- Trypsinate mES-cells in the usual procedure (e.g. 0.25% trypsin solution).
- Once the cells have become round and detach from the surface (the process can be speeded at 37 °C), resuspend them in DPBS by thoroughly pipetting up and down several times and centrifuge for 5 min at 180g at room temperature.
- Evacuate supernatant sterile and remove it.
- Resuspend cell pellet in DPBS again by pipetting up and down several times.
- Good dissociation of the cells is important, the goal being to obtain single cells or very small aggregates of only 2-3 cells. Larger cell clumps should not remain.
- Due to the serum-free formulation of PowerStem ESPro1, there is no trypsin inactivating effect of FBS; use trypsin-inhibitor to stop trypsin activity.
- Count cells and plate them on gelatine-coated culture dishes in PowerStem ESPro1
- Incubate the cells in the usual way in an incubator at 37 °C and 5% CO_2.
- Feed cells with fresh PowerStem ESPro1 every second day.
- The cells should be split at ratios between 1:4 and 1:8 depending on the growth rates.

Please note: For differentiation studies LIF supplement must be omitted.

mES-cells in PowerStem ESPro1



JM8-cells in PowerStem ESPro1

mES-cells in medium with 10% FBS

PowerStem ESPro 1 with LIF	100 ml Kit 500 ml Kit	P04-7701K P04-77010K
PowerStem ESPro 1 without LIF	100 ml Kit 500 ml Kit	P04-7751K P04-77510K

PowerStem ESPro2

PowerStem ESPro2 is a serum-free medium for cultivation and expansion of embryonic stem cells of mice (mES-cells). PowerStem ESPro2 is especially designed to proliferate and expand mouse ES-cells without differentiation. To differentiate the proliferated mouse ES-cells into different cell types the relevant protocols and differentiation factors can be used.

Content:
PowerStem ESPro2 medium consists of:

- PowerStem ESPro2 basal medium
- PowerStem ESPro2 growth supplement, which is added at the time of use
- PowerStem ESPro2 LIF supplement, which is added at the time of use

Storage conditions:

- PowerStem ESPro2 basal medium: store in the dark at +2 to +8 °C

PowerStem ESPro2

- PowerStem ESPro2 growth supplement: store in the dark at -20 °C
- PowerStem ESPro2 LIF supplement: store in the dark at -20 °C

Composition:
PowerStem ESPro2 contains purified proteins, lipids, salts, amino acids, trace elements, attachment factors, hormones and growth factors in an optimized formulation. PowerStem ESPro2 is fully chemically defined and contains no peptones or hydrolysates.

Please note: Supplemented PowerStem ESPro2 contains LIF in a concentration of 10 µg/L. If higher levels of LIF are required, please add additional LIF to the medium.

Suitability:
PowerStem ESPro2 is especially designed for the serum-free cultivation of murine embryonic stem cells (mES cells), while maintaining the undifferentiated state. PowerStem ESPro2 is suitable for the serum-free generation of knockout-mice from genetically modified mES-cells. PowerStem ESPro2 has also been proven to support the serum-free cultivation and expansion of tumor progenitor cells.

Please note: For research use only, not for therapeutic or diagnostic use.

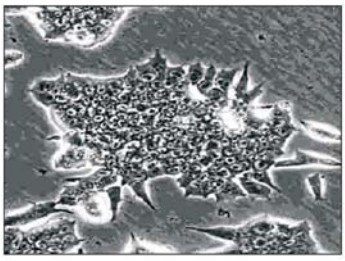
Special advantages:
PowerStem ESPro2 allows the cultivation and expansion of mouse embryonic stem cells (mES-cells) under serum-free conditions. It is fully defined in its composition and thus enables constant and comparable experimental conditions resulting in highly reproducible data. The mES-cell culture can be established without the usual feeder layer (primary fibroblasts), cells show a high proliferation rate and largely retain an undifferentiated state. By adding specific differentiation factors, mES-cells can differentiate in vitro to the desired cell types (e.g. nerve cells, muscle cells, endothelial cells, etc.).

Preparation of PowerStem ESPro2 medium:
PowerStem ESPro2 basal medium requires supplementation with PowerStem ESPro2 growth supplement and PowerStem ESPro2 LIF supplement. PowerStem ESPro2 complete medium (basal medium with supplement) is stable for 1 month when stored in the dark

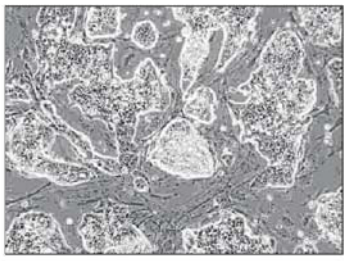
Instructions for use:

- Prepare gelatine-coated plates by covering them with a 0.2% gelatine solution for at least 10 min in the incubator.
- mES-cells should always be kept at a relatively high cell density to maintain their pluripotency.
- The subculture is best carried out from a sub-confluent culture (70%-80% confluence).
- Individual colonies should not touch each other.
- Too dense growth promotes the differentiation of cells and thus may cause the loss of pluripotency.
- Trypsinate mES-cells in the usual procedure (e.g. 0.25% trypsin solution).
- Once the cells have become round and detach from the surface (the process can be speeded at 37 °C), resuspend them in DPBS by thoroughly pipetting up and down several times and centrifuge for 5 min at 180x g at room temperature.
- Evacuate supernatant sterile and remove it.
- Resuspend cell pellet in DPBS again by pipetting up and down several times.
- Good dissociation of the cells is important, the goal being to obtain single cells or very small aggregates of only 2-3 cells. Larger cell clumps should not remain.
- Due to the serum-free formulation of PowerStem ESPro2, there is no trypsin inactivating effect of FBS; use trypsininhibitor to stop trypsin activity.
- Count cells and plate them on gelatine-coated culture dishes in supplemented PowerStem ESPro2
- Incubate the cells in the usual way in an incubator at 37 °C and 5% CO2.
- Feed cells with fresh PowerStem ESPro2 every second day.
- The cells should be split at ratios between 1:4 and 1:8 depending on the growth rates.

Please note: For differentiation studies LIF supplement must be omitted.



mES-cells in PowerStem ESPro2



mES-cells in PowerStem ESPro2

ES-cells in medium with 10% FBS

PowerStem ESPro 2 with LIF	100 ml Kit 500 ml Kit	P04-7702K P04-77020K
PowerStem ESPro 2 without LIF	100 ml Kit 500 ml Kit	P04-7762K P04-77620K

PowerStem HE1

PowerStem HE1 is a specialized serum-free medium for the cultivation and expansion of human embryonic stem cells (hES-cells) or induced pluripotent stem cells (iPScells). Pluripotent human embryonic stem cells or iPS-cells have the capacity to differentiate into all of the somatic cell types and therefore hold great promise for regenerative medicine. Even after long-term culture, cells maintained on Matrigel or Laminin retain a normal karyotype and a stable proliferating rate.

Content:
PowerStem HE1 medium consists of:

- PowerStem HE1 basal medium
- PowerStem HE1 growth supplement, which is added at the time of use.

Storage conditions:

- PowerStem HE1 basal medium: store in the dark at +2 to +8 °C
- PowerStem HE1 growth supplement: store in the dark at -20 °C

PowerStem HE1 basal medium and PowerStem HE1 growth supplement are guaranteed stable for 12 months when properly stored. PowerStem HE1 complete medium (basal + supplement) is stable for 1 month when stored in the dark at +2 to +8 °C. We do not recommend using the complete medium beyond 1 month.

Composition:
PowerStem HE1 contains purified proteins, lipids, salts, amino acids, trace elements, attachment factors, hormones and growth factors in an optimized formulation. PowerStem HE1 is fully chemically defined and contains no peptones or hydrolysates.

Please note: PowerStem HE1 contains b-FGF in a concentration of 20 µg/L. If higher b-FGF levels are required, please add additional b-FGF to the medium.

Suitability:
Serum-free cultivation of human embryonic stem cells (hES-cells) and induced pluripotent stem cells (iPS-cells), while maintaining an undifferentiated state.

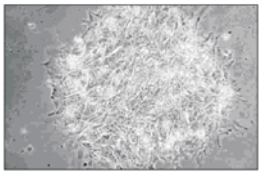
Please note: For research use only, not for therapeutic or diagnostic use.

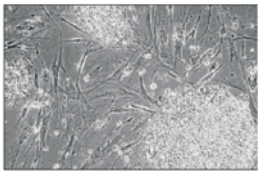
Special advantages:
PowerStem HE1 allows the cultivation and expansion of hES-cells and iPS-cells under serum-free conditions. It is fully defined in its composition and thus enables constant and comparable experimental conditions resulting in highly reproducible data. The hES- and iPS-cells can be cultivated without the usual feeder layers (primary fibroblasts), they show a high proliferation rate and largely retain their undifferentiated state. By adding specific differentiation factors, hES- and iPS-cells can differentiate in vitro to the desired cell types (e.g. nerve cells, muscle cells, endothelial cells, etc.).

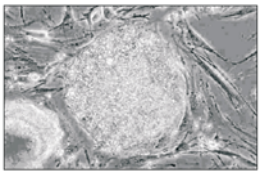
Preparation of PowerStem HE1 medium:
PowerStem HE1 basal medium requires supplementation with PowerStem HE1 growth supplement. Thaw PowerStem HE1 growth supplement before use. The thawed material should be used immediately or aliquoted and stored at -20 °C. To obtain 500 ml PowerStem HE1 complete medium please add 80 ml of thawed PowerStem HE1 growth supplement to 420 ml of PowerStem HE1 basal medium. PowerStem HE1 complete medium (basal medium with growth supplement) is stable for 1 month when stored in the dark at +2 °C to +8 °C.

Instructions for use:

- Prepare Fibronectin-coated plates by covering them with a Fibronectin stock solution (0.5 mg/10 ml of sterile water) for at least 30 min in the incubator. Recommended final concentration of Fibronectin: 50 µg/10 cm^2. If desired, Matrigel matrix can replace Fibronectin. The recommended dilution is 1:40. The matrix should be prepared according to the manufacturer's instructions.
- The starter culture must be a high quality culture and there must be a high density of undifferentiated cells. • The time of subculture is critical. Do not passage the cells too early, they will plate poorly and differentiate. The cultures need to grow to near-confluence.
- Individual colonies should not touch each other.
- Too dense growth promotes the differentiation of cells and thus the loss of pluripotency.
- Use Collagenase for passaging the cells. • Warm appropriate amount of Collagenase IV solution (10 mg/ml), wash medium (DPBS) and complete medium to 37 °C in a water bath.
- Aspirate the medium and add 1 to 2 ml collagenase to cover the cells.
- Leave for 3 minutes to dislodge cell colonies from substrate. Do not expose longer than 3 minutes. This will cause poor plating and may induce differentiation. • Add 3 ml of culture medium and gently collect cells with a 5 ml pipette.
- Collect cell suspension and put into conical tube and centrifuge for 5 min at 300x g at room temperature.
- Re-suspend cells in PowerStem HE1 and plate directly on fibronectin-covered plate.
- Cells should be split at a recommended ratio of 1:2 every 4-5 days. The cultures should be fed every day.


hES-cells in PowerStem HE1


hES-cells colony in PowerStem HE1


hES-cells in medium with 10 % FBS

PowerStem HE1	100 ml Kit	P04-7711K
	500 ml Kit	P04-77110K

PowerStem HE2

PowerStem HE2 is a specialized serum-free medium for cultivation and expansion of human embryonic stem cells (hES-cells) or induced pluripotent stem cells (iPS-cells). Pluripotent human embryonic stem cells or iPS-cells have the capacity to differentiate into all of the somatic cell types and therefore hold great promise for regenerative medicine. Even after long-term culture, cells maintained on Matrigel or Laminin retain a normal karyotype and a stable proliferating rate.

Content:
PowerStem HE2 medium consists of:

- PowerStem HE2 basal medium
- PowerStem HE2 growth supplement, which is added at the time of use.

Storage conditions:

- PowerStem HE2 basal medium: store in the dark at +2 °C to +8 °C
- PowerStem HE2 growth supplement: store in the dark at -20 °C

PowerStem HE2 basal medium and PowerStem HE2 growth supplement are guaranteed stable for 12 months when properly stored. PowerStem HE2 complete medium (basal + supplement) is stable for 1 month when stored in the dark at +2 °C to +8 °C. We do not recommend using the complete medium beyond 1 month.

Composition:
PowerStem HE2 contains purified proteins, lipids, salts, amino acids, trace elements, attachment factors, hormones and growth factors in an optimized formulation. PowerStem HE2 is fully chemically defined and contains no peptones or hydrolysates.

Please note: PowerStem HE2 contains b-FGF in a concentration of 2 µg/L. If higher b-FGF levels are required, please add additional b-FGF to the medium.

Suitability:
Serum-free cultivation of human embryonic stem cells (hES-cells) and induced pluripotent stem cells (iPS-cells), while maintaining an undifferentiated state.

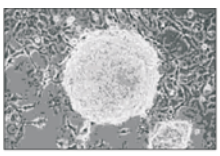
Please note: For research use only, not for therapeutic or diagnostic use.

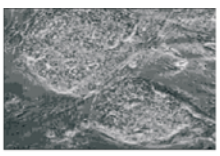
Special advantages:
PowerStem HE2 allows the cultivation and expansion of hES-cells and iPS-cells under serum-free conditions. It is fully defined in its composition thus enabling constant and comparable experimental conditions resulting in highly reproducible data. The hES- and iPS-cells can be cultivated without the usual feeder layers (primary fibroblasts), they show a high proliferation rate and largely retain their undifferentiated state. By adding specific differentiation factors, hES- and iPS-cells can differentiate in vitro to the desired cell types (e.g. nerve cells, muscle cells, endothelial cells, etc.).

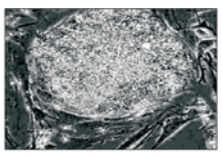
Preparation of PowerStem HE2 medium:
PowerStem HE2 basal medium requires supplementation with PowerStem HE2 growth supplement. Thaw PowerStem HE2 growth supplement before use. The thawed material should be used immediately or aliquoted and stored at - 20 °C. To obtain 500 ml PowerStem HE2 complete medium please add 80 ml of thawed PowerStem HE2 growth supplement to 420 ml of PowerStem HE2 basal medium. PowerStem HE2 complete medium (basal medium with growth supplement) is stable for 1 month when stored in the dark at +2 °C to +8 °C.

Instructions for use:

- Prepare Fibronectin-coated plates by covering them with a Fibronectin stock solution (0.5 mg/10 ml of sterile water) for at least 30 min in the incubator. Recommended final concentration of Fibronectin: 50 µg/10cm^2. If desired, Matrigel matrix can replace Fibronectin. The recommended dilution is 1:40. The matrix should be prepared according to the manufacturer's instructions.
- The starter culture must be a high quality culture and there must be a high density of undifferentiated cells.
- The time of subculture is critical. Do not passage the cells too early, they will plate poorly and differentiate. The cultures need to grow to near-confluence.
- Individual colonies should not touch each other.
- Too dense growth promotes the differentiation of cells and thus the loss of pluripotency.
- Use Collagenase for passaging the cells.
- Warm appropriate amount of Collagenase IV solution (10 mg/ml), wash medium (DPBS) and complete medium to 37 °C in a water bath.
- Aspirate the medium and add 1 to 2 ml collagenase to cover the cells.
- Leave for 3 minutes to dislodge cell colonies from substrate. Do not expose longer than 3 minutes. This will cause poor plating and may induce differentiation.
- Add 3 ml of culture medium and gently collect cells with a 5 ml pipette.
- Collect cell suspension and put into conical tube and centrifuge for 5 min at 300x g at room temperature.
- Re-suspend cells in PowerStem HE2 and plate directly on fibronectin-covered plate.
- Cells should be split at a recommended ratio of 1:2 every 4-5 days. The cultures should be fed every day.


hES-cells in PowerStem HE2


hES-cells in PowerStem HE2


hES-cells in medium with 10 % FBS

PowerStem HE2	100 ml Kit	P04-7712K
	500 ml Kit	P04-77120K

PowerStem EST

PowerStem EST is a serum-free system for the cultivation and proliferation of undifferentiated mouse embryonic stem cells (mES-cells) and their subsequent differentiation into beating myocardial cells (e.g. for the embryonic stem cell test EST). The EST has been formally validated by the European Centre for Validation of Alternative Methods (ECVAM) as an acceptable in vitro embryotoxicity assay. The in vitro embryonic stem cell test (EST) allows for categorisation of the embryotoxic potential of chemicals and drug candidates. For the screening process of newly developed chemicals and pharmaceuticals, a prediction model was developed based on the inhibition of differentiation of murine embryonic stem cells into cardiomyocytes. The application of the EST for chemical testing reduces time, testing costs and the amount of animal experimentation for embryotoxicity tests.

Composition:
PowerStem EST medium kit is composed of a complex basal medium containing salts, amino acids, vitamins, and micronutrients to which a serum-free supplement (PowerStem EST growth supplement) consisting of a mixture of proteins, growth factors and hormones is added immediately prior to use. For sustainment in undifferentiated condition and growth of ES cells, mouse leukemia inhibitory factor (mLIF, 1000 U/ml) is added to the supplemented basal medium (PowerStem EST LIFsupplement). For differentiation into beating myocardial cells, a mix of differentiation factors (PowerStem EST differentiation supplement) is added to the supplemented basal medium (without mLIF).

Suitability:
Cardiomyocytes differentiated from stem cells can be used for a multitude of purposes:

- Use in basic research for examining early development processes needed for functional cardiogenesis in vitro.
- Testing chemicals and pharmaceutical ingredients for mutagenicity, cytotoxicity and embryotoxicity (embryonic stem cell test, EST)
- Screening of anti-angiogenetic substances
- Electrophysiological analyses for investigating cardioactive drugs
- Development of new active ingredients

The basal medium is used for both, proliferation and differentiation; defined factors are added according to the objective – sustainment and growth or differentiation of ES cells.

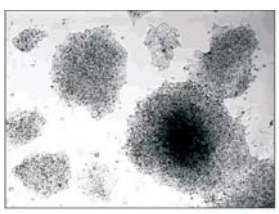
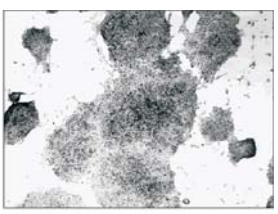
Special Advantages:
Traditionally, in vitro differentiation of mouse embryonic stem cells takes place using foetal bovine serum (FBS). It has been shown that the use of FBS is a limiting factor for successful differentiation of ES cells into cardiomyocytes. Some batches of FBS result in poor differentiation, while some batches may not allow differentiation at all. The search for suitable FBS batches and the dramatic variability makes the differentiation of ES cells with serum-containing media a time and money consuming exercise. In contrast, it has been demonstrated that the number of differentiated ES cells is substantially increased under serum-free conditions and the rate of differentiation is quite stable. The PowerStem EST medium kit successfully stimulates the expansion of undifferentiated ES-cells and promotes their subsequent differentiation into beating myocardial cells under serum-free conditions, resulting in highly comparable findings from standardized experiments.

Preparation:
Store supplemented PowerStem EST in a refrigerator at +2 °C to +8 °C (protected from light). After opening, the bottle should be used within one week. Any supplementing factors such as mLIF (Proliferation) as well as the differentiation supplement (Differentiation) are only added to the basal medium (supplemented with Growth Supplement) immediately before use. Please avoid repeated freeze-thaw cycles of supplements! Do not freeze complete media!

Instructions for use:
Adaption, cultivation and proliferation of undifferentiated ES cells
When initial culture was performed in serum containing medium, the embryonic stem cells are gradually adapted to the serum-free medium, which has been supplemented with Growth Supplement and LIF Supplement. In the process, the FBS concentration is slowly reduced (from passage to passage) and the concentration of PowerStem EST is gradually increased. By adding mLIF (1000U/ml), the cells are maintained in an undifferentiated state. Cell cultivation is performed in 0.1% gelatine-coated culture dishes.

Initial culture of ES cells
P1: 100% serum containing medium - 0% PowerStem EST
P2: 75% serum containing medium - 25% PowerStem EST
P3: 50% serum containing medium - 50% PowerStem EST
P4: 25% serum containing medium - 75% PowerStem EST
P5: 0% serum containing medium - 100% PowerStem EST

Differentiation assay procedure:
For differentiation, a mix of various differentiation factors (PowerStem EST differentiation supplement) is added to the supplemented basal medium. A detailed protocol the differentiation assay is provided with the accompanying data sheet for PowerStem EST.

ES cells cultivated in PowerStem EST with LIF (staining: alkaline phosphatase)

PowerStem EST	100 ml Kit	P04-77210K
	500 ml Kit	P04-77250K

PowerStem MSC1

PowerStem MSC1 is an easy to use xeno-free medium without animal derived components (ADCF) for cultivation and proliferation of human mesenchymal stem cells (hMSC). PowerStem MSC1 is especially designed for the proliferation of human mesenchymal stem cells without differentiation. PowerStem MSC1 supports long-term growth of MSC and preserves their multi-lineage potential. In addition, MSC cultured in PowerStem MSC1 expands faster and shows a significant reduction in hematopoietic cell contamination at early passages compared to serum-based media. To differentiate the proliferated MSC into different cells types the relevant protocols and differentiation factors should be used.

Composition:
PowerStem MSC1 medium consists of:

- PowerStem MSC1 basal medium
- PowerStem MSC1 growth supplement, which is added at the time of use.

Storage conditions:

- PowerStem MSC1 basal medium: Store in the dark at 2 - 8 °C
- PowerStem MSC1 growth supplement: Store in the dark at -20 °C (will be shipped on blue ice, should be used immediately on arrival or may be refrozen for later use)

Both the PowerStem MSC1 basal medium and PowerStem MSC1 growth supplement are guaranteed stable for 6 months when properly stored. PowerStem MSC1 complete medium (basal + supplement) is stable for 1 month when stored in the dark at 2 - 8 °C. We do not recommend using the complete medium beyond one month. Do not freeze complete PowerStem MSC1 medium.

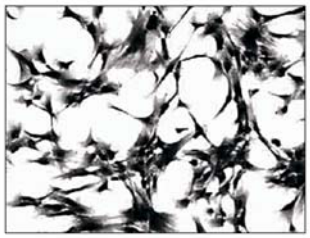
Composition:
PowerStem MSC1 contains salts, amino acids, trace elements, hormones, growth factors, and enriched human proteins and lipids in an optimized formulation. PowerStem MSC1 is free of animal derived components (ADCF, xenofree) and contains no undefined peptones or hydrolysates.

Suitability:
Serum-free cultivation of human mesenchymal stem cells (hMSC) while maintaining the undifferentiated state and multi-lineage potential. Please note: For research use only, not for therapeutic or diagnostic use.

Special advantages:
PowerStem MSC1 allows the cultivation of human mesenchymal stem cells under xeno-free conditions. It is free of animal or human serum and thus enables constant and comparable experimental conditions resulting in highly reproducible data. PowerStem MSC1 is completely free of animal components (ADCF, xeno-free) and thus suitable for a research approach in regenerative medicine and tissue engineering. By adding specific differentiation factors, MSC can differentiate in vitro to the desired cell types (bone, cartilage, adipose tissue etc.).

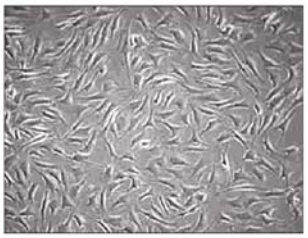
Preparation of PowerStem MSC1 medium:
PowerStem MSC1 basal medium requires supplementation with PowerStem MSC1 growth supplement. Thaw PowerStem MSC1 growth supplement before use. The thawed growth supplement should be added to the basal medium immediately. PowerStem MSC1 complete medium (basal medium with growth supplement) is stable for 1 month when stored in the dark at 2 - 8 °C.

Instructions for Use:
For detailed instructions please see instruction manual for isolation and culture of hMSC at www.pan-biotech.de

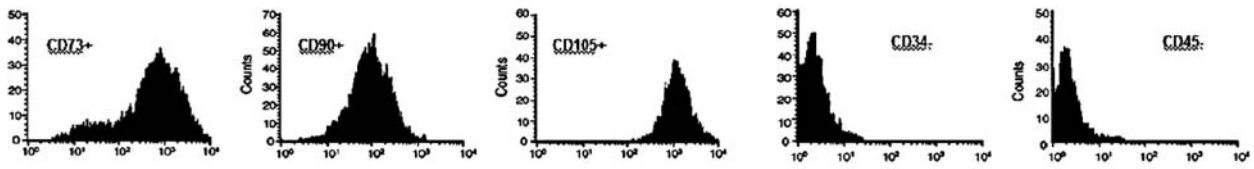


Sub-confluent hMSC in PowerStem MSC1

Confluent hMSC in PowerStem MSC1



hMSC in medium with 10% FBS



The culture-expanded cell population expresses CD90 (Thy-1), CD105 (SH2) and CD73 (SH3/SH4) but lacks expression of CD34 and CD45.

PowerStem MSC1	100 ml Kit	P04-77310K
	500 ml Kit	P04-77350K

PowerStem HPSC

PowerStem HPSC is a specialized serum-free medium for the cultivation and expansion of human hematopoietic stem cells (HPSC) and cells of myeloid lineage in suspension culture. Hematopoietic stem cells are CD34+, which are the earliest hematopoietic stem cells identifiable in bone marrow, peripheral blood and neonatal cord blood. By adding one or more differentiation factors or changing culturing conditions, HPSC can be induced to differentiate into different types of hematopoietic lineage cells.

Content:
PowerStem HPSC medium consists of:

- PowerStem HPSC basal medium
- PowerStem HPSC growth supplement, which is added at the time of use.
- PowerStem HPSC cytokine supplement, which is added at the time of use.

Storage conditions:

- PowerStem HPSC basal medium: store in the dark at +2 to +8 °C
- PowerStem HPSC growth supplement: store in the dark at -20 °C
- PowerStem HPSC cytokine supplement: store in the dark at -20 °C

PowerStem HPSC basal medium, PowerStem HPSC growth supplement and PowerStem HPSC cytokine supplement are guaranteed stable for 12 months when properly stored. PowerStem HPSC complete medium (basal + supplements) is stable for 3 months when stored in the dark at +2 °C to +8 °C. We do not recommend using the complete medium beyond 3 months.

Composition:
PowerStem HPSC contains purified proteins, lipids, salts, amino acids, trace elements, attachment factors, hormones and growth factors in an optimized formulation. PowerStem HPSC is fully chemically defined and contains no FBS or any other animal derived components.

Suitability:
Serum-free cultivation and expansion of human hematopoietic CD34+ stem cells from bone marrow, peripheral blood and neonatal cord blood.

Please note: For research use only, not for therapeutic or diagnostic use.

Special advantages:
PowerStem HPSC allows the cultivation and expansion of human hematopoietic CD34+ stem cells and cells of myeloid lineage under serum-free conditions. It is fully defined in its composition and thus enables constant and comparable experimental conditions with easily reproducible results. The hematopoietic stem cells can be cultivated without stromal cells, they show a high proliferation rate and largely retain their undifferentiated state. By adding specific differentiation factors, hematopoietic cells can be differentiated in vitro to different types of hematopoietic lineage cells.

Preparation of PowerStem HPSC medium:
PowerStem HPSC basal medium requires supplementation with PowerStem HPSC growth supplement and PowerStem HPSC cytokine supplement. Thaw PowerStem HPSC supplements before use. The thawed material should be used immediately or aliquoted and stored at -20 °C. To obtain 500 ml PowerStem HPSC complete medium please add 13 ml of thawed PowerStem HPSC growth supplement and 1 ml PowerStem cytokine supplement to 486 ml of PowerStem HPSC basal medium. PowerStem HPSC complete medium (basal medium with supplements) is stable for 3 months when stored in the dark at +2 °C to +8 °C.

Instructions for use:

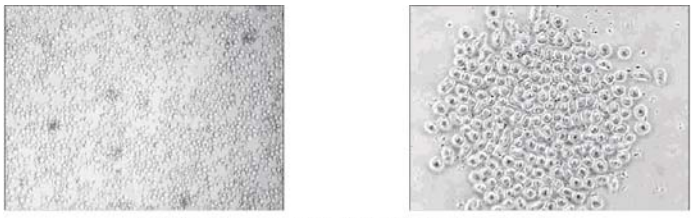
Expansion

- Prepare mononuclear cells (e.g. PBMC) with Pancoll human (P04-60500). For further enrichment of CD34+ cells use e.g. MiniMACS (Miltenyi) or comparable systems according to the manufacturer's instructions.
- CD34+ cells were seeded at an initial density of $2x10^4$ cells/ml in PowerStem HPSC complete medium at 37 °C in an incubator with 5% CO_2/95% air atmosphere.
- An initial lag phase of about 3 days is observed, because the majority of hematopoietic stem cells are in a quiescent (G0) state.
- Replace spent medium with fresh complete medium every 3-4 days.

Differentiation

- Erythropoietin (EPO) and Interleukin 6 (IL-6) stimulate the differentiation of CD34+ hematopoietic cells into red blood cell precursors (BFU-E cells, burst forming unit erythroid).
- For the development of BFU-E cells add EPO 10 U/ml and IL-6 10 µg/ml (final concentration) to the cell culture

Reference:
Horschitz S et al. (2010) Generation of neuronal cells from human peripheral blood mononuclear cells. NeuroReport 21:185



Hematopoetic stem cells from neonatal cord blood in PowerStem HPSC

PowerStem HPSC	100 ml Kit	P04-77410K
	500 ml Kit	P04-77450K

PowerStem PEC1

Endothelial cells line blood vessels and the internal cavities of the heart. They display a flattened, polygonal form and adhere to each other by desmosomes and tight-junctions. With a total number of about 1012 cells, the endothelium is one of the biggest organs of the body and plays a key role in many physiological and pathophysiological processes. A number of factors control proliferation and apoptosis of endothelial cells, thereby regulating maintenance, degeneration, or regeneration of blood vessels.

New blood vessel formation occurs via angiogenesis or vasculogenesis, a process restricted to embryonic development. In 1997, postnatal vasculogenesis has been proposed as an important mechanism for angiogenesis via blood or bone marrow derived circulating progenitor endothelial cells (PEC) (Asahara et al, Science 1997). PEC have been extensively studied as potential cell therapy for the repair of damaged blood vessels. Animal studies clearly demonstrated that administration of PEC partially rescued cardiovascular dysfuntion or myocardial injury with evidence for PEC contribution to new vessel growth.

While controversy exists as to the identity of endothelial cell progenitors, recently a PEC population has been identified which shows expression of typical endothelial as well as progenitor markers (Ingram et al, Blood. 2004;104:2752-2760). Importantly, these cells have been tested for a high proliferative potential in clonogenic assays and characterized by formation of functional blood vessels in vivo (Yoder et al, Blood. 2007;109:1801-1809).

With endothelial cell progenitors rapidly moving into the field of interest for vascular tissue engineering with potential therapeutic application, the presence of whole animal serum or animal-derived components in culture media is undesirable for a cell therapeutic approach.

Product Description:

PowerStem PEC1 *ready-to-use* (P04-777500) is a specially developed medium for a serum- and xeno-free in vitro culture of human progenitor endothelial cells (hPEC) containing all components necessary for optimal colony formation, clonogenic growth, and rapid proliferation. It is designed for use in an incubator at 37 °C with a 5% CO_2 atmosphere. Please avoid repeated warming of complete medium. Prepare only the amount needed in a separate sterile tube. For a T25 cell culture flask it is recommended to use 5 ml of PowerStem PEC1. For smaller or larger culture area, please adjust volume accordingly. When cells have been thawed, change the medium after 24 h to remove un-attached cells; for maintenance and propagation, change the medium every two or three days; for cultures close to confluence or for maximum proliferative response, it is recommended to use more medium or more frequent changes. Store at 2 - 8 °C in the dark. Expiry: 3 months.

PowerStem PEC1 *kit* (P04-77750K) is provided with supplements (pre-screened and tested for progenitor cells) in separate sterile packing. This will enable the user to prepare a medium for special application. For example, VEGF, FGF-2, or other components may be omitted from the complete medium for specific experimental settings. Please note that such a formulation will not promote optimal cell growth. Therefore, this composition can not be used for routine long-term culture of PEC. Please make sure that sterility is not compromised when adding individual components to prepare complete medium. The medium should be carefully but thoroughly mixed after addition of all components to assure a homogeneous solution. Store basal or complete medium at 2 - 8 °C and store supplements at -20 °C. Expiry: 6 months.

Basal medium (w/o supplements) or complete/ready-to-use medium should not be frozen!

WARNING: PowerStem PEC1 is not suited for stopping trypsin reaction. Please use trypsin inhibitor solution (P10-033100) to neutralize trypsin. To avoid damage to cells, progenitor endothelial cells should be exposed only for a minimum period of time to trypsin.

Product intended use: FOR RESEARCH USE ONLY!
Not approved for human or animal diagnostic or therapeutic procedures.

PowerStem PEC1 is suitable for the culture of:
Human Umbilical Cord Blood Progenitor Endothelial Cells
Human Adult Peripheral Blood Progenitor Endothelial Cells
Human Bone Marrow-derived Progenitor Endothelial Cells

Quality Control:
Each batch of PowerStem PEC1 is tested for its proliferation promoting capacity on freshly isolated primary human cord blood progenitor endothelial cells to guarantee optimum performance and reliability. In addition, the medium is tested for absence of microbial contamination (bacteria, yeast, fungi, mycoplasma).

References:

a) Asahara T et al. (1997) Isolation of putative progenitor endothelial cells for angiogenesis. Science 275:964

b) Ingram DA et al. (2004) Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. Blood 104:2752

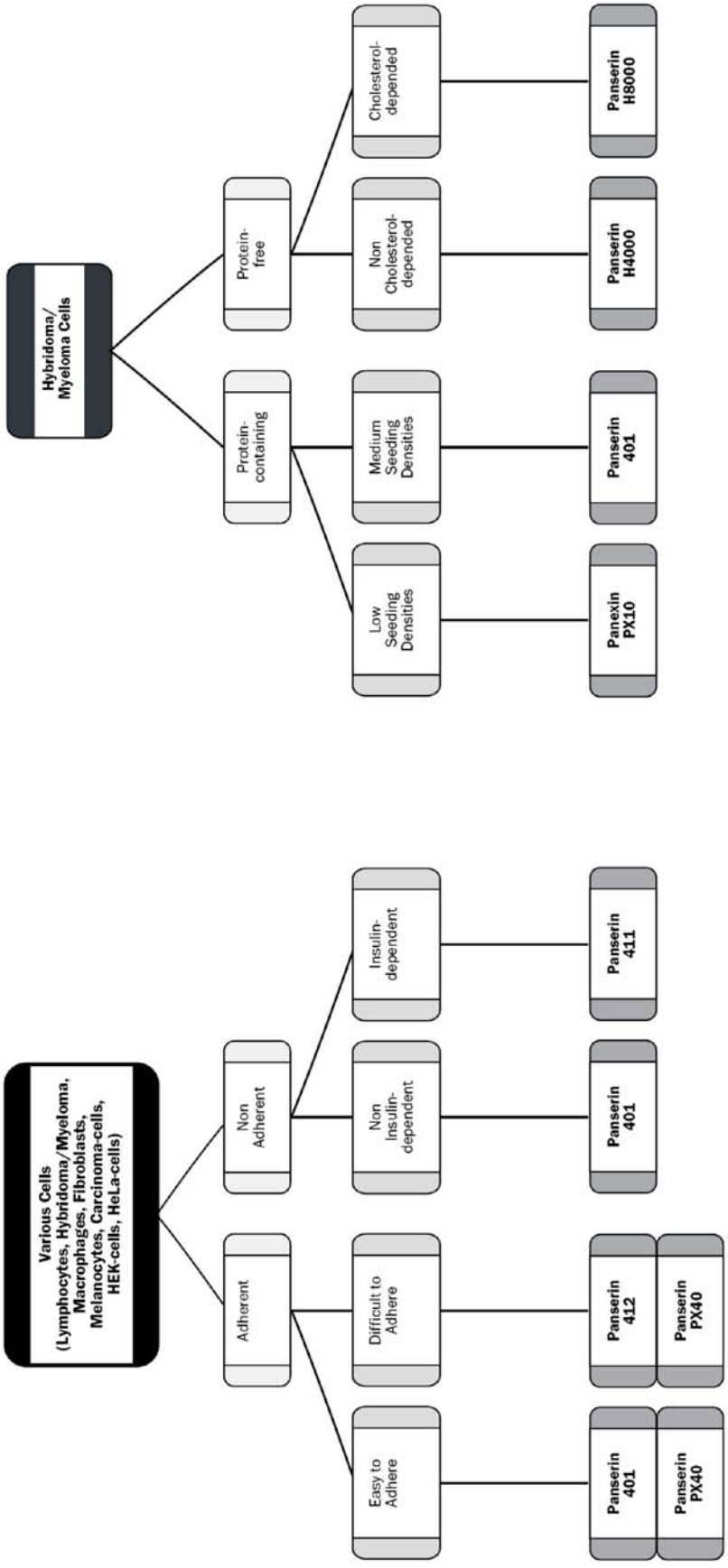
c) Yoder MC et al. (2007) Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. Blood 109:1801

For more references see www.pan-biotech.de.

PowerStem PEC1 ready-to-use	500 ml	P04-777500
PowerStem PEC1 kit	500 ml	P04-77750K

Decision Tree

Decision Tree 1



Decision Tree

Decision Tree 2