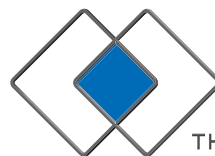


Cell Culture Media

QUALITY, RELIABILITY, INDIVIDUALITY



PAA

THE CELL CULTURE COMPANY



PAA | The Cell Culture Company

This brochure is designed for everyone who does cell culture in the daily work. We would like to give a deeper insight into the fascinating and multifaceted world of cell culture media. Beside historical backgrounds this brochure will provide detailed information about different media formulations and useful hints about the correct supplementation.



For 20 years PAA is a leading manufacturer of media, buffers, reagents and sera for cell culture. In the course of these 20 years, cell biological research experienced many important breakthroughs, developed new techniques and resulted in application on an industrial scale. As part of this process PAA developed special media for newly-established cell lines. In times of BSE we research in the field of serum-free cell culture and produce reagents for the innovative techniques of the so-called Advanced Therapies. In the area of cell culture, established media are improved to support the work in research and biopharmaceutical production efficiently.

Our state-of-the-art manufacturing plants guarantee the high quality standard of our culture media and thus the success of your cell culture.

Cell culture media are produced in our state-of-the-art GMP¹-compliant manufacturing plant in Pasching / Austria. All raw materials are pre-tested and have to conform to the most stringent quality standards. Media production takes place according to GMP production guidelines (SOP²). Only purified water (AP grade) is used. Controls of important parameters such as pH, osmolality and endotoxin are performed prior to sterile filtration. The media are bottled on fully automatic filling lines. A release of our products occurs only after the successful completion of a comprehensive quality control. Certificates of Analysis and full documentation can be requested for each individual batch.

We focus on customer service and are dedicated to prove this every day in meeting your specific demands. When your project requirements go beyond our standard catalogue products we are able to produce customized media or other products in individual packaging sizes.

¹Good Manufacturing Practice

²Standard Operating Procedures

Media | The Living Space for Cells

In the laboratory cell culture media have to simulate the individual living conditions of the cell *in vitro*. In addition, they should not impede the scientific objectives but rather support the experimental workflows. To meet all these demands the composition of the media can be extremely complex and is a result of long time experience and development work. PAA uses its expert knowledge for the production of high-quality media.

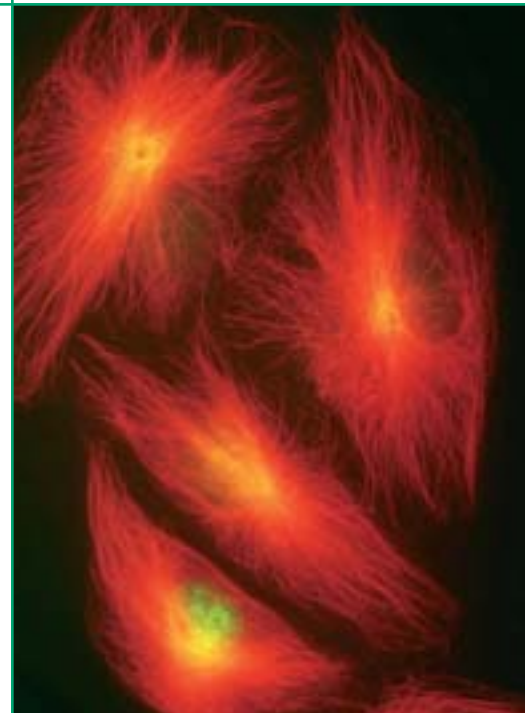
The cell culture media provide an optimal pH value, specific osmolality and nutrients, such as vitamins and trace elements. If required special growth and differentiation factors are included. It takes up metabolic products, neutralizes and buffers. Therefore, the culture medium offers the cell an optimum surrounding. Sterility is another very important condition for successful work in cell culture because for germs, a culture medium is a blessing as well.

Animal cells make the highest demands on the medium because, unlike bacteria or yeasts, they have no protective cell wall and are separated from the surrounding medium by a double membrane only. Therefore, animal cells are particularly delicate, and often react very sensitive to suboptimal culture conditions.

In contrast to bacteria and yeasts, animal cells do not proliferate undefined even if there are sufficient nutrients available in the medium. In fact their proliferation is regulated by growth factors which have to be added to the medium. The life expectancy of primary cells decreases with advancing cell division, because at each mitotic division the telomer sequences at the chromosomes become shorter. Are the sequences expended, the cell goes into senescence, the cell-death. The cell ceases its division and dies. Therefore, tumor cell lines are commonly used in cell culture, which are no longer subject to this limitation and reproduce indefinitely.

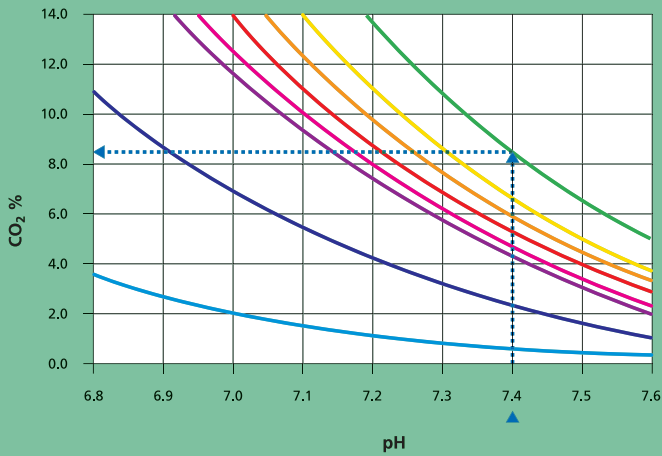
Last but not least, high quality culture media must satisfy the requirements of experimental studies. Is the aim an increase in cell mass, or the optimum production of a DNA or a protein? Does the composition of the medium for example make the later isolation of an antibody more difficult? Is the end product affected by ingredients of the medium? ...

PAA can meet the individual needs of different fields of research with a broad range of media.



Cultivated in the right medium cells develop structures as in the tissue they originate from.

Media | The Essential Components



NaHCO ₃ (g/l)	Medium	%CO ₂ (pH 7.4)
3.70	DMEM	8,5
3.02	ReadyMix DMEM	
3.02	IMDM	7.0
2.75	Glasgow's MEM	6.0
2.45	DMEM / Ham's F12	5.5
2.20	MEM with Earle's Salts	5.0
2.00	Medium 199 with Earle's Salts	
2.00	MEM Alpha modification	
2.00	RPMI 1640	4.5
2.00	ReadyMix RPMI	
1.20	Ham's F10	2.5
1.17	Ham's F12	2.5
0.35	MEM with Hanks' Salts	0.5

The pH value of the medium depends on the CO₂ partial pressure of the ambient atmosphere.

SALTS

The osmolality of the medium is just as critical as the pH value. It indicates the concentration of osmotic active salts dissolved in the medium which regulate the water balance of the cell. Depending on the cell line this lies between 260 and 340 mOsmol/kg. The salts used supply the cells at the same time with important minerals and trace elements, for example sodium chloride, potassium chloride, calcium chloride, iron nitrate, iron sulphate, magnesium sulphate, magnesium chloride or zinc sulphate. Calcium affects the growth behavior of many cells, that is why media are also provided without calcium. In keratinocytes for example differentiation is induced by calcium.

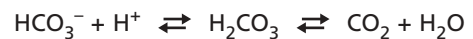
NUTRIENTS

To meet cells energy requirements they need energy-rich substances which they aerobically oxidize to CO₂. Usually **Glucose** is added to the medium in a defined concentration for this purpose. **Pyruvate** also acts as an energy source. The anabolic metabolism of the cells is dependent on amino acids, nucleotides, lipids, vitamins and trace elements in the medium. None of these components may be absent, if the cells are to proliferate in culture or to synthesize proteins.

BUFFER SUBSTANCES

Cultivated cells react extremely sensitive to variations in the pH value of the medium. Therefore, each medium contains suitable buffer substances which ensure an optimum pH value.

Sodium bicarbonate is used as a standard puffer. The puffer is equilibrated with the CO₂ contained in the atmosphere according to the following formula:



For this reason, the atmosphere in the incubator must have a controlled CO₂ content. The ratio between the CO₂ content and the pH value in media containing different amounts of sodium bicarbonate is shown in the opposite diagram.

HEPES is a potassium salt which has a stronger buffer action than bicarbonate. It is a buffer substance also commonly used in culture medium. However, HEPES can be toxic for some cell lines, particularly when the cells are exposed to ultraviolet light.

Phenol red is very often added to media as a pH indicator, which is red at a pH of 7.4 and changes its colour to orange or yellow as the pH decreases. This change in colour makes it much easier to control the conditions inside the cell culture flasks. However, in certain applications, e.g. fluorescence microscopy or hormone-free cell culture, phenol red can interfere. Therefore PAA provides many culture media without phenol red.

Supplements | That Extra Something

There are hundreds of substances which can be added to a culture medium. However, supplements in the narrower sense support the establishment and maintenance of cell cultures. Supplements can be chemically defined substances as well as natural products as sera or tissue extracts like bovine pituitary extract (BPE). We would like to give you an overview about the most important supplements below and explain the supplements used for different purpose. More detailed information can be found from page 15 onwards.

SERUM

The growth of animal cells depends crucially on the growth factors contained in the medium. In most cases this is achieved by adding blood serum to the medium which also contains proteins, vitamins, hormones, trace elements and adhesion factors. The most commonly used sera are of bovine origin. The strongest growth-enhancing action is obtained with fetal serum, as it contains the highest concentration of growth factors and the lowest concentration of antibodies. However, sera from other animals such as horse as well as human sera have also a broad range of applications. The vast number of sera available would go beyond the scope of this booklet. We would like to refer at this point to the PAA brochure "**Sera for Cell Culture**", which can be requested free of charge at www.paa.com. There you will find everything worth knowing about the different types of sera and their use in cell culture.

SERUM PROTEINS

Serum proteins such as BSA are used in cell culture, immunohistochemistry and in blot- and ELISA-analyses. In cell culture, it serves e.g. as a nutrient and undertakes carrier function through the cell membrane and binds toxic metabolic products.

GROWTH FACTORS, HORMONES, CYTOKINES

This very heterogeneous substance group includes steroids, aromatic amino acid compounds, polypeptides, proteins and glycoproteins. They influence the differentiation, morphogenesis, proliferation or activation of the cells and play an important role when serum is not added. Some well-known examples are:

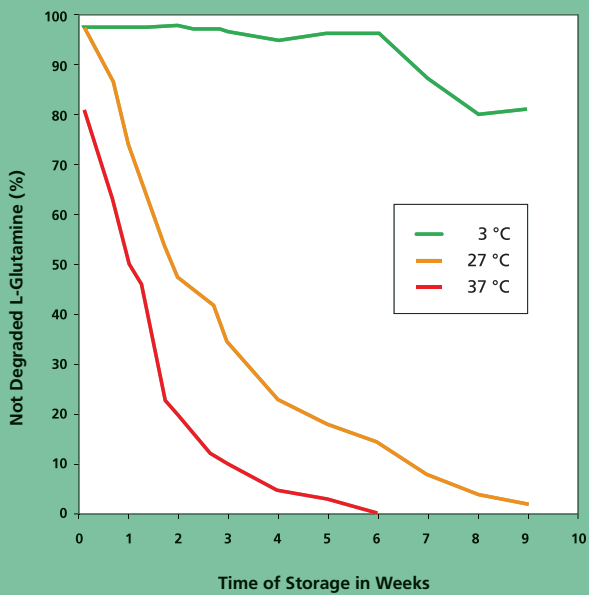
- Basic fibroblast growth factor (bFGF),
- insulin-like growth factor (IGF-1),
- mitosis-inducing phytohaemagglutinin (PHA).

Finding the right composition for a particular cell line is a highly complex procedure. Therefore, PAA offers a comprehensive range of cell type-specific ready to use media, which already contain the correct amount of factors as well as pre-tested supplement mixtures for special cell lines. This includes the

- Hybridoma Cloning Supplement (HCS),
- Insulin-Transferrin-Selenium (ITS),
- products for neuronal cell culture such as • B27 NeuroMix, • G5 and
- N2 Supplement.



Comprehensive information about sera can be found in our brochure "**Sera for Cell Culture**".



L-Glutamine is a temperature-sensitive amino acid which degrades rapidly.

AMINO ACIDS

There are only 20 different amino acids in all living organisms. Animal cells are not able to synthesize all of them by themselves. Thus, so-called essential amino acids must be present in the culture medium. In addition, certain cell lines have lost the capability to produce non essential amino acids. Using defined amino acid concentrates they can be added to the culture media.

A special media option can be the substitution of **L-Valin** by **D-Valin**. Such a medium inhibits the growth of rapidly proliferating fibroblasts when isolating epithelial cells from tissue samples.

GLUTAMINE

The essential amino acid **L-Glutamine** has the property of being relatively thermolabile. The degradation of L-Glutamine leads to NH_4^+ , which damages the cell membranes and can be toxic for particularly sensitive cells. Therefore, it can be an advantage to add L-Glutamine to the media directly before use. The opposite diagram shows the temperature-dependent degradation of L-Glutamine in the medium. Alternatively, **stable Glutamine (L-alanyl-L-glutamine)** can be used which is metabolized in the same way as L-Glutamine by most mammalian cell lines.

VITAMINS

Vitamins make up a very inhomogeneous substance group. They all have in common that they act as co-enzymes or co-substrates in cell metabolism. They cannot be synthesized by the cells and are therefore an essential component of culture media. Cells that should grow over a longer period of time or reach a particularly high density require media supplemented with additional vitamins. Normally vitamin concentrates are used (e.g. **MEM Vitamins**).

ANTIBIOTICS & ANTIMYCOTICS

Antibiotics and antimycotics selectively suppress the growth of bacteria and fungi. A special combination of different antibiotics kills mycoplasma in cell culture (e.g. **MycoKill AB**). These substances are on no account a substitute for sterile operation. However, they can be very useful when establishing a primary culture for which cells are taken from only partially sterilized tissues. The use of **Penicillin**, **Streptomycin**, **Gentamicin** and **Amphotericin B** is widespread.

SELECTIVE ANTIBIOTICS

In contrast to the antibiotics named above, which are harmless for eukaryotic cells, selective antibiotics have a toxic action on all cells which have no resistance. They are used exclusively for the selection of successfully transfected cells. External DNA is cloned into a vector which as a marker confers resistance to the relevant selective antibiotic. Transfected cells are resistant to these selective antibiotics and grow in their presence. Popular selective antibiotics are **G-418 Sulphate**, **Hygromycin B**, **Blasticidin** and **Puromycin**. **Carbenicillin** is used in special methods for the genetic modification of dicotyledonous plant cells.

Standard Media | The Classics

To this day, the father of cell culture is considered to be Ross Harrison, who in 1907 first succeeded in preserving spinal cord tissue from the frog for several weeks in frog lymph. It was not until 41 years later, in 1948, that the first chemically defined cell culture medium was developed in the laboratory of Fisher. He named it **CMRL 1066** (Connaught Medical Research Laboratories), and it is still used today for certain virological and cytological studies.

In 1950 Morgan, Morton and Parker described the first fully defined culture medium. It was known by the name of **Medium 199 (M199)** and used for the cultivation of primary embryonic chicken heart cells. Nowadays it is mainly used in virus and vaccine production.

Harry Eagle was the first to conduct systematic research on the nutritional requirements of cultivated cells and developed, based on these findings, **Eagle's Basal Medium (EBM)** in 1955 which was specially designed to meet the needs of HeLa cells. This was followed in 1959 by **Eagle's Minimum Essential Medium (MEM)**. This is suitable for the culture of a variety of cell types in monolayer and is widely used to this day.

In the same year Dulbecco and Freeman presented a modified MEM (**Dulbecco's modified Eagle's Medium, DMEM**), that contains higher concentrations of amino acids, vitamins and glucose. The medium was originally intended for the cultivation of embryonic mouse cells and is used nowadays for a variety of cell lines, including liver cells and endothelial cells.

In 1958 McCoy *et al.* established on basis of BME Medium **McCoy's 5A Medium** with an optimized amino acid concentration for Novikoff hepatoma cells. Today it is used for a multiplicity of different mouse, rat and human cell lines.

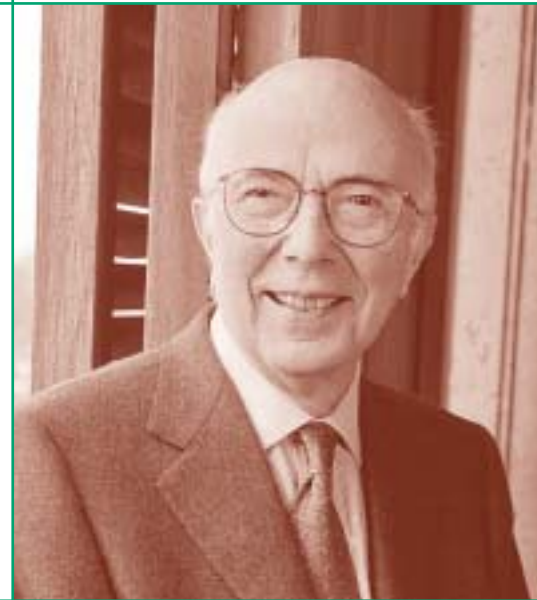
In 1971 Stanners *et al.* presented the so-called Alpha Modification of MEM (**MEM-Alpha Modification**). This medium does not contain nucleotides and was used for the culture of hamster kidney cells. Nowadays it finds its application in embryonic cell culture and supports the development of cells to the morula and blastula stage.

In 1976 Norman Iscove added selenium, sodium pyruvate, other amino acids, vitamins and HEPES to DMEM. This enriched medium supports the growth of many blood and bone marrow cell lines and is known under the name **Iscove's modified DMEM (IMDM)**. Supplemented with serum, it is suitable for many cell types, including lymphocytes or hybridoma cells.

Another popular modification of MEM was developed in 1967 by Moor *et al.* in the Rosewell Park Memorial Institute (**RPMI 1640**). Originally intended for the cultivation of leukaemia cells, it is nowadays mainly used as a medium for lymphocytes, but also for a broad spectrum of other mammalian cell lines.

For serum-free cultivation of Chinese hamster ovary cells (CHO) Ham, another pioneer in the field of cell culture, developed a medium which became known by the name of **Ham's F-10**. It is widely used for primary cultures of rabbit or chicken cells, but also for chromosome analysis. Supplemented with serum, it is used today for a variety of mammalian cell types and hybridoma cells. A modification of this medium is **Ham's F-12** which was originally used in serum-free culture of CHO cells. Supplemented with serum it can be used for the cultivation of various other mammalian cell lines, in particular of rat and rabbit hepatocytes and prostate cells. The combination of DMEM with Ham's F-12 (**DMEM/Ham's F-12**) was first described in the context of studies on the hormone requirements of cultivated cells. This composition is characterized by a particularly efficient buffer system.

Leibovitz worked on the effect of the ambient atmosphere on the growth of cultivated cells. He developed a medium for these studies whose buffer system is effective without bicarbonate buffers and thus without CO₂ in the incubator. The pH value in **Leibovitz L-15** is buffered by other salts, basic amino acids (e.g. L-Arginine) and galactose.



Renato Dulbecco, a pioneer in cell culture, developed Dulbecco's Modified Eagle Medium (DMEM) in 1955 and received the Nobel Prize in Medicine in 1975

DMEM & RPMI 1640

Dulbecco's Modified Eagle Medium (DMEM) and RPMI 1640 are the most commonly used media in cell culture. Like almost all media from PAA, both are offered with and without the amino acid L-Glutamine, or the stable Glutamine dipeptide L-alanyl-L-glutamine (see p. 6). For particularly pH-sensitive cells, we offer them with 25 mM HEPES or for fluorescence microscopic applications without the addition of phenol red.

In the original formula, DMEM contains glucose in a concentration of 1 g/litre. In addition to this "low glucose" DMEM a "high glucose" DMEM is available which contains 4.5 g glucose/litre and supports cells with a higher energy demand. For cells with an individual glucose requirement, we recommend glucose-free DMEM which can be supplemented with the desired quantity of glucose under sterile conditions. We also supply DMEM with pyruvate or without calcium e.g. for calcium-sensitive keratinocyte cultures. A DMEM in which L-Valin is substituted by its enantiomer D-Valin blocks the proliferation of fibroblasts and is used mainly in the primary culture of epithelial cells.

SILAC DMEM

SILAC DMEM is a cell culture medium developed for stable isotope labelling with amino acids (SILAC). This method supports the analysis of protein-protein-interactions and relies on the incorporation of isotope-labelled amino acids. SILAC DMEM contains neither Lysine nor Arginine. These amino acids have to be added before use.

THERMOSTABLE MEDIA

PAA can supply DMEM and RPMI 1640 in a thermostable form. Due to special production processes thermolabile media components such as vitamins and minerals are protected by synthetic clathrate molecules. These thermostable media can be stored at room temperature for up to 12 months.

READY MIX

The use of Ready Mix media, which already contain 10% FBS GOLD, is particularly convenient and easy. FBS GOLD is a fully defined FBS, which makes batch testing and reservation unnecessary. DMEM and RPMI 1640 Ready Mix can be used at once, without further supplementation in the cell culture.

MEM & MEDIUM 199

PAA supplies MEM with Earle's salts with or without L-Glutamine. The alpha modification of MEM without nucleotides is also part of our product range.

Medium 199 is available either supplemented with Earle's or with Hanks' Salts.

IMDM & L-15

Iscove's modification of DMEM and Leibovitz's L-15 medium, both with and without L-Glutamine are included in the PAA product portfolio.

HAM'S F-10 & HAM'S F-12

PAA manufactures both media introduced by Ham, either with or without L-Glutamine.

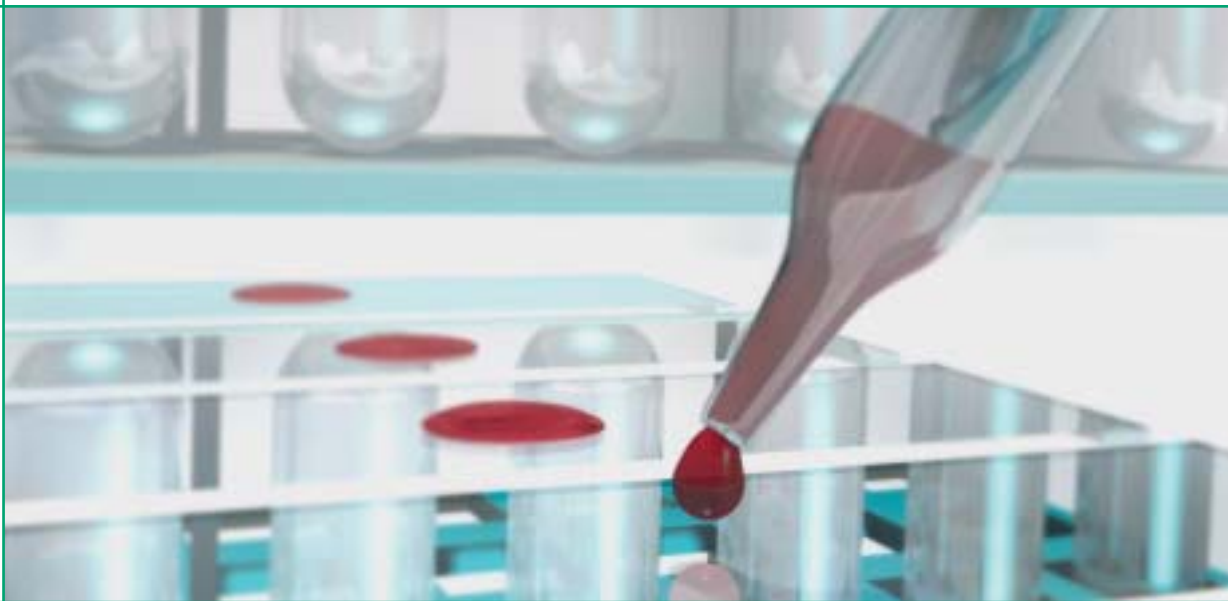
McCOY'S 5A

Originally developed for liver carcinoma cells, this medium is suitable for a broad range of cells from the mouse, rat and human system. PAA offers McCoy's 5A medium without L-Glutamine.



Special Media | For Cytogenetics

In addition to classical media, PAA manufactures a wide range of special media. They are optimized for the individual requirements of cell types. This may involve specially supplemented standard media or completely new developed formulations. In addition to this cell type-specific composition, some media also contain components which are tailored to suit particular fields and applications.



MEDIA FOR CYTOGENETIC APPLICATIONS

Quantum 3-21

Chorionic villus sampling and amniocentesis are important prenatal diagnostic procedures. This involves isolating and cultivating cells from the amniotic cavity and testing the chromosomes for abnormalities. An important condition for the successful cultivation of chorionic villi and amnion cells is the use of pretested complete media.

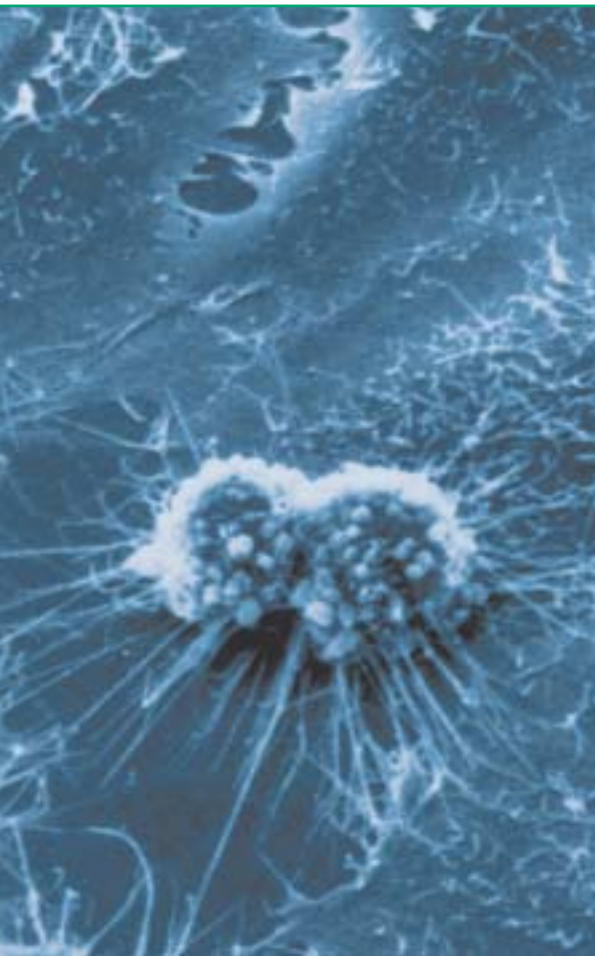
Working in collaboration with several human genetics institutes, PAA has developed a new complete medium. The addition of special growth factors led to a significant improvement of the growth properties of amnion and chorionic villi cells. The innovative media formulation allows rapid growth, by inhibiting other unwanted cell types.

Using Quantum 3-21, clear karyograms can be drawn to detect possible chromosomal abnormalities.

Quantum PBL

Karyotyping is an important tool in cytogenetics to detect chromosomal abnormalities. For cytogenetic analysis peripheral blood lymphocytes are cultivated and then used for chromosome analysis.

PAA offers with Quantum PBL a complete medium for the selective growth of lymphocytes of the peripheral blood. It is composed of a modified RPMI 1640 medium, phytohaemagglutinin (PHA) and a FBS pretested on lymphocytes. Along with the FBS, the mitogen PHA is one of the growth determining factors. Through comprehensive testing the concentration was optimized for the stimulation of lymphocytes. Sufficient cells are available for preparation of intact chromosomes. Clear karyograms are produced for detection of possible chromosomal abnormalities.



MEDIA FOR STEM CELLS

Stem cells are undifferentiated cells with the potential for self-renewal. Depending on endogenous stimuli, they are able to convert into any cell in the body. Modern medicine is looking at stem cells to provide a wide range of groundbreaking therapeutic opportunities for diseases which are at present not curable. This makes the cultivation of stem cells one of the most important tasks of many research institutions.

MethoStem Medium

This special medium has been developed for the cultivation of hematopoietic stem cells. It is based on IMDM, where methylcellulose, BSA, 2-mercaptoethanol, and L-Glutamine are added. The methylcellulose increases the viscosity and improves the colony formation of the cells. To support its growth and differentiation, the medium has to be supplemented with 10 – 30% FBS and cytokines before use.

MesenchymStem Medium

Mesenchymal stem cells are derived from bone marrow. They are capable to differentiate into bone, cartilage, muscle and fat cells. Differentiated cells can be used for tissue repair *in vivo*. MesenchymStem Medium from PAA is a DMEM base medium, supplemented with FBS, L-Glutamine and trace elements. For cell differentiation, cytokines must be added to the medium.

MEDIA FOR SPECIAL CELL TYPES

Endothelial Cell Medium

Endothelial cells are a popular model system to study the vascular system, the production of growth factors or the metabolism of lipids. Endothelial cell medium is serum free and fully defined. It is also available as a kit including the individual components Endothelial Basal Medium MCDB 131 and Endothelial Medium Supplement.

Macrophage Medium Murine

Macrophages and monocytes are an important *ex vivo* model system for research of immunological processes. The PAA Macrophage Medium is optimized for the cultivation of these cell types derived from mice. It is a fully defined, serum-free medium, which does not induce any unspecific cell stimulation.

Hybridoma Express & Hybridoma Express Plus

Hybridoma cells are usually cultivated in standard media with FBS. The Hybridoma Express Media from PAA have a low protein content and support the growth of cells without the addition of serum. This guarantees high antibody production and facilitates the isolation and purification of the antibodies. The media are also suitable for use in the fermenter, since they contain Pluronic F68 for minimizing the occurring shear forces.

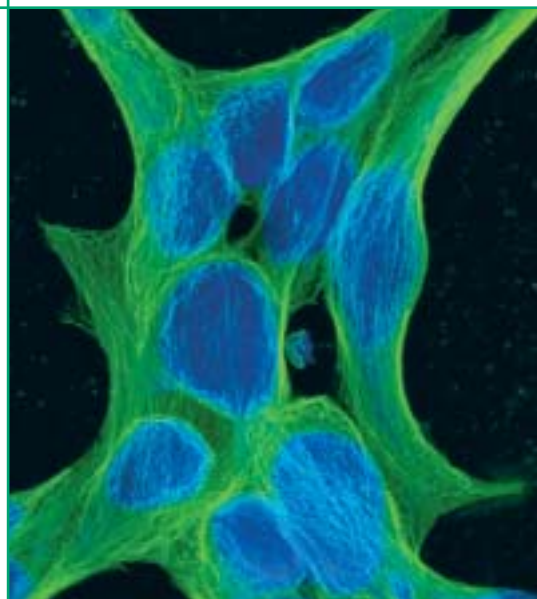
InsectExpress Sf9-S2, TC 100 Insect Medium & Insect Express Prime

InsectExpress Sf9-S2 and Insect Express Prime Medium are serum and protein free. Therefore, they are particularly suitable for the effective baculovirus expression system, as they do not impede the detection of synthesized proteins. The media are suitable for the cultivation of *Spodoptera frugiperda* and *Drosophila* S2 cells and can be used for adherent cells and for suspension cultures.

TC 100 Insect Medium is a modification of the Grace Medium. It is tested on many insect cell lines and is particularly suitable for *lepidoptera* cells, Sf9 cells and the baculovirus expression system. A supplementation with growth factors is recommended.

QUANTUM MEDIA

Quantum Media from PAA are newly formulated complete media, which do not have to be supplemented with serum. Their cell type specific composition with growth factors, vitamins and trace elements guarantees optimal conditions even for sensitive cells.



Quantum 101, Medium for HeLa Cells

Based on RPMI 1640 and supplemented with chromatographically purified growth factors and components of yeast and soybean extract, this medium supports particularly the growth of HeLa cells.

Quantum 263, Medium for Tumor Cells

Tumor cells have an unlimited ability to divide and are thus potentially immortal. This makes them suitable for long-term cultivation. Quantum 263 is based on RPMI 1640. It is supplemented with special trace elements, yeast extract and selected serum components and provides ideal cell culture conditions for tumor cells. Quantum 263 contains a synthetic iron binding molecule, which facilitates the uptake of iron ions into the cell.

Quantum 286, Medium for Epithelial Cells

Epithelial cells play an important role in the control of resorption and secretion in tissue. Quantum 286 is a modified formulation based on DMEM, supplemented with selected serum components, trace elements and protein fractions from yeast and soy beans. It supports the proliferation of epithelial cells, without influencing the characteristic physiological processes of these cells.

Quantum 333, Medium for Fibroblasts

Fibroblasts play an important role in the recovery of injured tissues. Quantum 333 is a base medium especially developed for the fibroblasts cell culture, supplemented with a balanced combination of growth factors, trace elements and serum components. In addition, Quantum 333 contains insulin and a synthetic iron binding molecule.

Quantum 724, Medium for Primary Lymphocytes

Lymphocytes are part of the cellular fraction of the blood and belong to the adaptive immune system. Adult lymphocytes lack the ability to proliferate. Thus the medium has to contain a mitogen to induce cell division. Quantum 724 is a complete medium for the cultivation of lymphocytes from peripheral blood. It consists of a modified RPMI 1640 and a lymphocyte pretested FBS.

Media | A Wide Range of Choice

DMEM, 1 g/l Glucose	Cat. No.	Volume
DMEM, without L-Glutamine	E15-005	500 ml
DMEM, with L-Glutamine	E15-806	500 ml
DMEM, with 25 mM HEPES, without L-Glutamine	E15-007	500 ml
DMEM, with 25 mM HEPES, with L-Glutamine	E15-808	500 ml
DMEM, thermostable medium, without L-Glutamine	E15-080	500 ml

DMEM, 4,5 g/l Glucose	Cat. No.	Volume
DMEM, without L-Glutamine	E15-009	500 ml
DMEM, with L-Glutamine	E15-810	500 ml
DMEM, with stable Glutamine	E15-883	500 ml
DMEM, with Sodium Pyruvate, without L-Glutamine	E15-011	500 ml
DMEM, with Sodium Pyruvate, with L-Glutamine	E15-843	500 ml
DMEM, without Phenol red, without L-Glutamine	E15-047	500 ml
DMEM, without Phenol red, with L-Glutamine	E15-877	500 ml
DMEM, with D-Valine, without L-Valine, without L-Glutamine	E15-055	500 ml
DMEM, without Calcium, without L-Glutamine	E15-078	500 ml
DMEM, without Glucose, without L-Glutamine	E15-079	500 ml
DMEM, thermostable medium, without L-Glutamine	E15-082	500 ml
Ready Mix DMEM, 4,5 g/l Glucose	R15-801	500 ml

DMEM/Ham's F-12	Cat. No.	Volume
DMEM/Ham's F-12, without L-Glutamine	E15-012	500 ml
DMEM/Ham's F-12, with L-Glutamine	E15-813	500 ml

SILAC DMEM	Cat. No.	Volume
SILAC DMEM, without Arginine, without Lysine, without L-Glutamine	E15-086	500 ml

Ham's F-10	Cat. No.	Volume
Ham's F-10, without L-Glutamine	E15-014	500 ml
Ham's F-10, with L-Glutamine	E15-815	500 ml

Ham's F-12	Cat. No.	Volume
Ham's F-12, without L-Glutamine	E15-016	500 ml
Ham's F-12, with L-Glutamine	E15-817	500 ml

Iscoves modified DMEM (IMDM)	Cat. No.	Volume
IMDM, with 25 mM HEPES, without L-Glutamine	E15-018	500 ml
IMDM, with 25 mM HEPES, with L-Glutamine	E15-819	500 ml

Leibovitz's L-15	Cat. No.	Volume
Leibovitz's L-15, without L-Glutamine	E15-020	500 ml
Leibovitz's L-15, with L-Glutamine	E15-821	500 ml

McCoy's 5A	Cat. No.	Volume
McCoy's 5A Medium, without L-Glutamine	E15-022	500 ml

Medium 199 with Earle's Salts	Cat. No.	Volume
Medium 199, with Earle's Salts, without L-Glutamine	E15-033	500 ml
Medium 199, with Earle's Salts, with L-Glutamine	E15-834	500 ml

Medium 199 with Hanks' Salts	Cat. No.	Volume
Medium 199, with Hanks' Salts, with HEPES, without L-Glutamine	E15-037	500 ml
Medium 199, with Hanks' Salts, with HEPES, with L-Glutamine	E15-838	500 ml
MEM Alpha Modification	Cat. No.	Volume
MEM Alpha Mod., without Ribonucleosides, with L-Glutamine	E15-832	500 ml
MEM Alpha Mod., with Ribonucleosides, with L-Glutamine	E15-862	500 ml
MEM with Earle's Salts	Cat. No.	Volume
MEM, with Earle's Salts, without L-Glutamine	E15-024	500 ml
MEM, with Earle's Salts, with L-Glutamine	E15-825	500 ml
RPMI 1640	Cat. No.	Volume
RPMI 1640, without L-Glutamine	E15-039	500 ml
RPMI 1640, with L-Glutamine	E15-840	500 ml
RPMI 1640, with stable Glutamine	E15-885	500 ml
RPMI 1640, with 25 mM HEPES, without L-Glutamine	E15-041	500 ml
RPMI 1640, with 25 mM HEPES, with L-Glutamine	E15-842	500 ml
RPMI 1640, without Phenol red, without L-Glutamine	E15-048	500 ml
RPMI 1640, without Phenol Red, with L-Glutamine	E15-848	500 ml
RPMI 1640, thermostable medium, without L-Glutamine	E15-084	500 ml
Ready Mix RPMI 1640	R15-802	500 ml
Media for Cytogenetic Applications	Cat. No.	Volume
Quantum 3-21, medium for cultivation of amnion cells and chorionic villi, with L-Glutamine	U11-020	100 ml
Quantum PBL, medium for Blood Lymphocytes, with L-Glutamine	U11-022	100 ml
Media for Stem Cells	Cat. No.	Volume
MethoStem Medium, with L-Glutamine	U11-827	100 ml
MesenchymStem Medium, with L-Glutamine	U15-828	500 ml
Cell Type Specific Media	Cat. No.	Volume
Endothelial Cell Medium, without L-Glutamine	U15-002	500 ml
Endothelial Basal Medium MCDB 131, without L-Glutamine	U15-011	500 ml
Endothelial Medium Supplement, with growth factors & heparin, without antibiotics	U05-012	50 ml
Macrophage Medium murine, without L-Glutamine	U15-005	500 ml
Hybridoma Express, without L-Glutamine	U15-001	500 ml
Hybridoma Express Plus, without L-Glutamine	U15-040	500 ml
InsectExpress Sf9-S2, with L-Glutamine	E15-875	500 ml
InsectExpress Prime, with L-Glutamine	E15-895	500 ml
	E21-895	1000 ml
TC 100 Insect Medium, with L-Glutamine	E15-856	500 ml
Quantum 101, Medium for HeLa Cells, with L-Glutamine	U15-814	500 ml
Quantum 263, Medium for Tumour Cells, with L-Glutamine	U15-815	500 ml
Quantum 286, Medium for Epithelial Cells, with L-Glutamine	U15-818	500 ml
Quantum 333, Medium for Fibroblasts, with L-Glutamine	U15-813	500 ml
Quantum 724, Medium for Primary Lymphocytes, with L-Glutamine	U11-841	100 ml

Media | Recommendations for Use

CELL TYPE	SUITABLE MEDIA
Amnion cells and Chorionic villi	Ham's F-10, Quantum 3-21
B-Lymphocytes (murine)	IMDM
Blood Lymphocytes	Quantum PBL, Quantum 724
Bone cells	Ham's F-12
Bone marrow (haematopoetic tissue)	IMDM
Bone marrow (primary culture)	DMEM
CHO cells	Ham's F-12, Ham's F-10
Chondrocytes	Ham's F-12, L-15 Medium
Endothelial cells	DMEM, Endothelial Medium
Epithelial cells	Medium 199 with Earle's or Hanks' Salts, Quantum 286
Erythrocytes progenitor cells	IMDM
Fibroblasts (mammalian)	Minimal Essential Medium, Eagle (MEM), Quantum 333
HeLa cells	RPMI 1640, Quantum 101
HeLa cells (subtypes)	Minimal Essential Medium, Eagle (MEM), Quantum 101
Haematopoetic progenitor cells (human and murine)	CollagenStem Kit
Hybridoma cells	Hybridoma Express, Hybridoma Express Plus
Insect cells	Insect Express Sf9, Insect Express Prime, TC 100 Insect Medium
Leucocytes (human)	RPMI 1640
Leydig cells and Sertoli cells	DMEM/Ham's F-12
Lymphoblast-like cells	RPMI 1640
Liver cells (murine)	DMEM
Macrophage progenitor cells	IMDM
Macrophages (murine)	Macrophage Medium
Mesenchymal stem- and Progenitor cells	MesenchymStem Medium
Monocytes	Macrophage Medium
Murine cells	Ham's F-10, Ham's F-12
Murine L-cells	Ham's F-12, Ham's F-10
Muscle cells	DMEM, Ham's F-12, DMEM/Ham's F-12
Myelocytes	RPMI 1640
Neuronal cells	L-15 Medium, DMEM/Ham's F-12
Pancreatic cells	DMEM/Ham's F-12
Primary explants (chicken, murine)	Ham's F-12
Primary explants (embryonic and adult tissue)	L-15 Medium
T-Lymphocytes (murine)	IMDM
Tumor cells (adherent)	DMEM, DMEM/Ham's F-12, Quantum 263
Tumor cells (suspension)	RPMI 1640, Quantum 263

Supplements | The Extra Something

AMINO ACIDS & VITAMINS

MEM Amino Acid Concentrate

This concentrate is used to ensure that cells are adequately supplied with essential amino acids. It completes Eagle's MEM or other media for cells with high protein expression.

NEAA Amino Acid Concentrate

PAA provides this important supplement for cells which have lost the ability to synthesize non essential amino acids. It is also suitable for strongly proliferating cells.

L-Glutamine & Stable Glutamine

Decay products of degraded L-Glutamine damage the membranes of cells. For certain cell lines it is sometimes advisable to add the temperature-sensitive essential amino acid immediately before use. L-Glutamine is also available as a ready to use solution with Penicillin and Streptomycin. The dipeptide L-Alanyl-L-Glutamine is thermostable and metabolized by most cells in the same way as L-Glutamine. It is therefore an alternative to the supplementation with the temperature-sensitive L-Glutamine.

MEM Vitamins

The 100x concentrate of this vitamin mixture provides the cells with co-enzymes and co-factors.

ANTIBIOTICS

Amphotericin B

This antifungal agent is used in tissue culture. It affects the cell membranes of fungal and yeast cells and forms channels, causing the loss of cations from these cells. Together with the inhibition of a number of membrane proteins, this leads to the die off of yeasts and fungi. Amphotericin B is ineffective for viruses and bacteria.

Antibiotics/Antimycotics

This antibiotic/antimycotic solution has a broad antifungal spectrum. It contains Penicillin, Streptomycin and Amphotericin B.

Gentamicin

This aminoglycoside binds to the 30S subunit of the bacterial ribosome and thus impedes protein biosynthesis. It is effective against gram negative and gram positive bacteria and facilitates the establishment of primary cultures.

Penicillin/Streptomycin

The broadband antibiotic inhibits the protein biosynthesis and the cell wall synthesis of gram positive and gram negative bacteria. It is used routinely in the cell culture as prophylaxis.

MycoKill AB

MycoKill AB is a highly effective antibiotic that inhibits protein synthesis and the transcription of known mycoplasma subspecies and thus effectively removes all mycoplasma from the cell culture.

Contaminations with mycoplasma are difficult to detect. The new **MycoTrace** kit by PAA allows the quick and reliable detection of mycoplasma by PCR technology.





SELECTIVE ANTIBIOTICS

G-418 Sulphate, Hygromycin B, Puromycin, Blastidicin, Carbenicillin

These antibiotics are used for the selection of successfully transfected cells. Co-transfected genes on the vector act as markers, which confer resistance to the relevant antibiotic.

GROWTH SUPPLEMENTS

Hybridoma Cloning Supplement (HCS)

Hybridoma Cloning Supplement (HCS) is a fully defined supplement. It contains hybridoma specific growth factors to obtain high cell densities for optimal cloning efficiency in the presence of serum within the medium. For increased production rates HCS can also be used as an enhancer for hybridoma cultivation in serum free conditions. The low protein content of HCS facilitates the isolation and purification of the produced antibodies.

B27 NeuroMix

B27 is a serum free supplement for long-term viability and growth of neurons of the central and peripheral nervous system as well as the hippocampus. The supplement is a chemically defined 50x concentrate.

G5 Supplement

Based on Bottenstein's G5 formulation, this defined supplement supports the growth of primary glial cells or tumor cells of astrocyte like phenotype.

N2 Supplement

The modification of Bottenstein's N2 formulation is ideal for the cultivation of neuroblastomas or primary cells from both the peripheral nervous system (PNS) and the central nervous system (CNS).

Neuronal Stem Cell Supplement

This serum-free supplement enhances the growth of neuronal stem cells without the need of specific feeder cells.

Insulin-Transferrin-Selenium (ITS)

ITS is used as a growth supplement and describes a mixture of human recombinant insulin, bovine transferrin and sodium selenite. It can be applied as a complete or partial replacement for fetal bovine serum (FBS) in the cell culture media.

ADDITIVES

Sodium Pyruvate

Sodium Pyruvate is an easily accessible carbohydrate source for strong proliferating cells. It is directly available for ATP synthesis.

Additives for Cytogenetics

These substances are used for the successful detection and preparation of metaphase chromosomes.

Giemsa Solution is a stain for visualizing the characteristic G-banding of the metaphase chromosomes. It is used for the identification of chromosomal abnormalities.

Potassium chloride is a hypotonic solution and causes the cells to swell and aids in the release of intact chromosomes.

Colcemid is a synthetic analogue of Colchicin but less toxic. It depolymerizes microtubules, thus inactivating the spindle fibre mechanism and keeps the cells in metaphase.

Phytohaemagglutinin (PHA) is a lectin from *Phaseolus vulgaris*. It induces mitotic cell division and increases proliferation in lymphocyte cultures. PAA provides a sterile ready to use solution of PHA with a defined concentration to avoid batch variations.

Supplements | by PAA

Amino Acids & Vitamins	Cat. No.	Volume
MEM Amino Acid Concentrate (50x)	M11-002	100 ml
NEAA Amino Acid Concentrate (100x)	M11-003	100 ml
L-Glutamine (200 mM)	M11-004	100 ml
Stable Glutamine (200 mM)	M11-006	100 ml
L-Glutamine with Penicillin/Streptomycin (100x)	P11-013	100 ml
MEM Vitamins (100x)	N11-002	100 ml

Antibiotics	Cat. No.	Volume
Amphotericin B	P11-001	100 ml
Antibiotic/Antimycotic Solution	P11-002	100 ml
Gentamicin (10 mg/ml)	P11-004	100 ml
Gentamicin (50 mg/ml)	P11-005	100 ml
Penicillin/Streptomycin (100x)	P11-010	100 ml
Mycokill AB (50x)	P11-016	100 ml

Growth Factors	Cat. No.	Volume
Hybridoma Cloning Supplement	F05-009	50 ml
B27 NeuroMix (50x)	F01-002	10 ml
G5 Supplement (100x)	F001-003	1 ml
N2 Supplement (100x)	F005-004	5 ml
Neuronal Stem Cell Supplement (50x)	F01-005	10 ml
Bovine Pituitary Extract (BPE)	F001-006	1 ml
	F050-006	5 x 1 ml
Insulin-Transferrin-Selenium (ITS)	F01-015	10 ml

Selective Antibiotics	Cat. No.	Volume
G-418 Sulphate	P21-011	1 g
	P25-011	5 g
	P31-011	10 g
G-418 Sulphate Solution (50 mg/ml)	P02-012	20 ml
	P11-012	100 ml
Hygromycin B	P15-014	500 mg
	P21-014	1 g
Hygromycin B Solution (50 mg/ml)	P02-015	20 ml
Puromycin	P11-019	100 mg
	P15-019	500 mg
Blasticidin	P05-017	50 mg
	P11-017	100 mg
Carbenicillin	P21-020	1 g
	P25-020	5 g
	P31-020	10 g

Other Reagents	Cat. No.	Volume
Sodium Pyruvate (100 mM)	S11-003	100 ml
Giemsa Stain Solution	S11-019	100 ml
Potassium Chloride (0,075 M)	S11-018	100 ml
Colcemid	J01-003	10 ml
Phytohaemagglutinin (PHA)	J01-006	10 ml

Media | Practical Tips

The manufacture of a ready to use medium carries a risk of various potential errors, which can jeopardize the success of your cell culture. Carelessness when handling the media leads to contamination, which cannot be reversed. Commercially available media often have to be supplemented with antibiotics, serum or L-Glutamine. Here the utmost caution is required, since even minor errors can destroy many months of work.



- ▶ Add serum, antibiotics and other required supplements under sterile conditions immediately before using the medium.
- ▶ Thaw serum and L-Glutamine rapidly and take the quantities required. Aliquot the remaining volume and freeze immediately.
- ▶ Transfer the required quantities of serum, L-Glutamine, antibiotics and other supplements under the sterile work bench with a new pipette each time.
- ▶ Mix the medium by swirling carefully. Equilibrate the medium in the incubator.
- ▶ Remove an aliquot of the medium under sterile conditions and measure the pH value. Adjust the optimal pH-value for your cells with sterile 1 N NaOH or 1 N HCl .
- ▶ Carry out a sterility test and do not use the medium before the analysis of the sterility is completed.

STORAGE

- ▶ Store the completed medium in the refrigerator at 2 – 8 °C and protect from light.
- ▶ Use liquid media with thermolabile components as quickly as possible.
- ▶ If liquid media containing Glutamine are stored for longer periods, the concentration of L-Glutamine may be reduced. Do not try to compensate this by the addition of Glutamine. Ammonia occurs when Glutamine degrades and acts as a cytotoxin in higher concentrations.

STERILE OPERATIONS

- ▶ Only use defined media and sera from reliable manufacturers.
- ▶ Take out partial quantities with the pipette. Avoid extraction by shaking, as the screw thread of the bottle may carry contaminants.
- ▶ Avoid the formation of foam by shaking or vacuum filtration, as proteins may denature. In addition, CO₂ is extracted from the medium, which shifts the pH value of the medium into the basic range. The risk of contamination increases if the foam reaches the neck of the bottle.
- ▶ In sterile filtration, pressure filtration (e.g. with a hose pump or an autoclave with N₂) is preferable to vacuum filtration, on account of the potential formation of foam.
- ▶ After sterile filtration, check the integrity of the filter using a pressure control test or a bubble point test.

Service

We Can Do More for You



PAA offers you not only a comprehensive ordering and delivery service. On request, we can also manufacture media to your own formulation. In addition to popular culture media with minor modifications, we also manufacture media for specific metabolic requirements e.g. with specific concentrations of glucose, amino acids or growth factors. The production of complex media, exactly to suit your requirements, is self-evident also part of our service. For biopharmaceutical applications we can supply media which are free from components of animal origin.

The minimum volume requirement for these products is 10 litres and lead time, including all QC/QA testing is normally between 6 to 8 weeks.

Give us a call, we will be pleased to advise you!



Our mission is not only to manufacture high-quality products for your cell culture, but also to support you with help and advice in your everyday scientific work. To achieve this goal PAA has a technical service team which offers you qualified, uncomplicated help if you have any questions on the subject of cell culture. You can reach our team at techservice@paa.com.

If you require detailed information on media or other products from PAA, you will find specific data about composition and physical-chemical properties in our main catalogue or in the Internet at www.paa.com.

A background image showing a microscopic view of cells, likely in culture, with a green tint. The cells are in various stages of division or growth, with some showing distinct nuclei and membranes. A thin green vertical line is positioned on the right side of the page, and a thin green horizontal line intersects it, creating a crosshair effect.

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