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Life Technologies™
Your Molecular & Cell Technology Partner

Serum Free and Animal Component Free Media And Reagents

Vero Cells

In the light of the growing worldwide shortage and consequent price instability associated with foetal bovine serum, the development of effective serum-free medium formulations has become essential for the future growth of the biotechnology industries. Batch variation in FBS requires prior sampling of each lot. Also the use of FBS in production of biologicals causes downstream purification difficulties.

NutriVero™ VP

Product Name	Catalogue No.	Unit Size	Storage Temp.
NutriVero™ VP1, Animal Component-Free Serum-Free Medium for the Monolayer Culture of Vero Cells (NutriVero™ VP1, ACF SFM)	05-066-1A 05-066-1B	500ml 100ml	2-8ºC 2-8ºC
NutriVero™ VP2, Animal Component-Free Serum-Free Medium for the Microcarrier Suspension Culture of Vero Cells (NutriVero™ VP2, ACF SFM)	05-067-1A 05-067-1B	500ml 100ml	2-8ºC 2-8ºC

A chemically defined, animal and human component-free serum-free medium, designed to support the growth of Vero cells used in virology, virus production, and biotechnology.

There are many problems associated with the use of animal sera e.g. the fear of contamination with viral agents such as BSE, Hepatitis, HIV, BVD or other potential adventitious agents. The culture of cells in serum-free and animal component-free medium eliminates those risks. Furthermore, it allows cells to be grown under a defined set of conditions.

NutriVero™ VP1 and NutriVero™ VP2 are serum free, very low protein media containing no proteins or peptides of human or animal origin.
NutriVero™ VP1 - designed specifically for monolayer culture of Vero cells.
NutriVero™ VP2 - designed specifically for microcarriers suspension culture of Vero cells. NutriVero™ VP1 and NutriVero™ VP2 are both suitable for large scale culturing and for growing viruses, as well as other cell culture applications, including production of recombinant proteins. The medium contains EGF and does not contain L-glutamine.

Features
- Very low protein concentration.
- No proteins or peptides of animal or human origin.
- The proteins that are used are human recombinant EGF and human recombinant Insulin.
- The formulation is without any animal origin components.
- Reduced risk of viral contamination.
- Lot to lot consistency.
- Ease of downstream product purification.

Quality Control
NutriVero™ VP1 and NutriVero™ VP2 are performance tested using Vero cells pre-adapted to serum-free culture in NutriVero™ VP1 and NutriVero™ VP2 correspondently. Additional standard evaluations are pH, osmolality and sterility tests.

Figure 1: Growth of Vero cells with NutriVero™ VP2 in microcarriers suspension culture (Cytodex-1) in spinner flask; cell counting performed using crystal violet nuclei staining method.

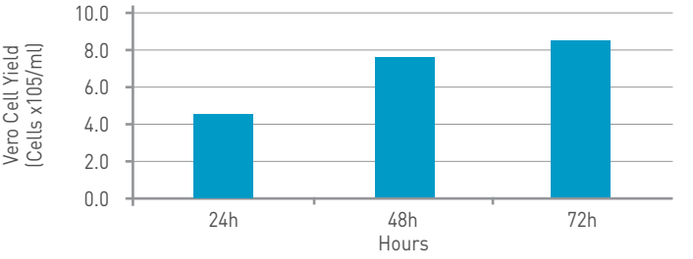
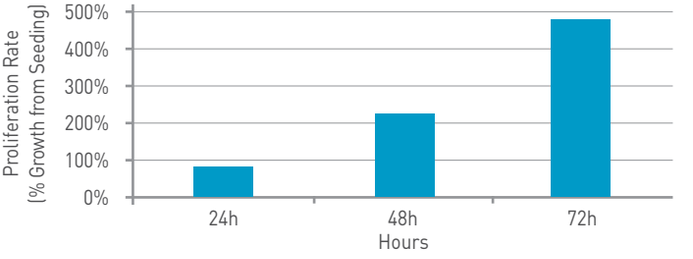


Figure 2: Growth of Vero cells with NutriVero™ VP2 in Bioreactor.



Hybridoma Cells

Biological Industries offers a series of serum-free media products for the growth of cells in suspension.

DCCM-1, DCCM-2, LPM, BIOGRO-1 & BIOGRO-2

Product Name	Catalogue No.	Unit Size	Storage Temp.
DCCM-1 Without L-Glutamine	05-010-1A 05-010-1B	500ml 100ml	2-8ºC 2-8ºC
DCCM-1 10X Conc. Without L-Glutamine Without Sodium Bicarbonate	05-010-5B	100ml	2-8ºC
DCCM-2 Without L-Glutamine	05-015-1A 05-015-1B	500ml 100ml	2-8ºC 2-8ºC
DCCM-2 10X Conc. Without L-Glutamine, Without Sodium Bicarbonate	05-015-5A 05-015-5B	500ml 100ml	2-8ºC 2-8ºC
Low Protein Media BSA-Free (LPM) Without L-Glutamine	05-040-1A 05-040-1B	500ml 100ml	2-8ºC 2-8ºC
Low Protein Media BSA-Free (LPM) **10X Conc.** Without L-Glutamine Without Sodium Bicarbonate	05-040-5B	100ml	2-8ºC
BIOGRO-1 Serum-Free Medium Supplement 50X Conc.	05-600-1B 05-600-1C 05-600-1D 05-600-1T	100ml 20ml 10ml 2ml	-20ºC -20ºC -20ºC -20ºC
BIOGRO-2 Serum-Free Medium Supplement 50X Conc.	05-610-1B 05-610-1C 05-610-1D 05-610-1T	100ml 20ml 10ml 2ml	-20ºC -20ºC -20ºC -20ºC

Applications

These formulations have been successfully used in all of the following cell culture applications:

- Culture of myeloma and hybridoma cells.
- Monoclonal antibody production.
- Culture of human lymphocytes cells (including stimulated or transformed cells).

The formulations of DCCM-1 and DCCM-2 contain no growth factors and are therefore cost efficient. The relatively higher protein content of DCCM-1 is aimed at maximizing cell growth, while the lower protein content in DCCM-2 represents a compromise between cell growth promotion and easier purification in monoclonal antibody production.

LPM Medium is a formulation totally free of bovine serum albumin. The protein content is therefore less than 18 micrograms per ml. Despite this very low protein content LPM has proven to be very effective for the growth of a wide variety of hybridomas and other lymphocytes.

BIOGRO-1 and BIOGRO-2 are serum-free supplements intended for those customers who prefer to prepare their own final medium using a basal medium of their choice.

Method of Use

DCCM-1, DCCM-2 and LPM are ready-to-use media. They only require the addition of L-Glutamine and antibiotics.
The BIOGRO products are 50 fold concentrates and therefore are recommended for use at a concentration of 2% (although in some cases 1% may be sufficient). DMEM: F-12 (1:1) has been found to be most generally effective as the basal media, but many cell lines grow well with RPMI, DMEM or Iscove's. Glutamine and antibiotics should be added to the final formulation.

Adaptation of Cells

For many cell types no adaptation procedures are necessary and may even be detrimental. In other cases standard-weaning procedures may be necessary.

Recommended Amounts of Sodium Bicarbonate and L-Glutamine to be Added in the Preparation of Single Strength Liquid Media (1x) from Concentrated Media (10x)

Product Name	Desired Product (1x) Cat. No.	Prepared from Product (10x) Cat. No.	Quantity Sodium Bicarbonate Solution 7.5% Cat. No. 03-040-1 ml/Liter	Quantity L-Glutamine Solution 200mM Cat. No. 03-020-1 ml/Liter
DCCM-1	05-010-1	05-010-5	29.4	10-20
DCCM-2	05-015-1	05-015-5	29.4	10-20
Low Protein Media BSA-Free (LPM)	05-040-1	05-040-5	29.4	10-20

Anchorage-Dependant Cells

A successful transition from cell culture work utilizing serum-containing media to serum-free cell culture often requires the use of techniques which were specifically developed for this purpose. For example, special techniques for trypsinization, neutralization of trypsin, cryopreservation of cells, as well as the use of an effective serum-free growth medium are all essential.

BIO-MPM-1, BIOCHO-1, BIOCHO-2 and BIOGRO-CHO have been successfully used in adherent and suspension cultures.

BIO-MPM-1 Multi-Purpose Serum-Free Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIO-MPM-1,Multi-Purpose SFM Without L-Glutamine	05-060-1A 05-060-1B	500ml 100ml	2-8ºC 2-8ºC

Bio-MPM-1 is a ready-to-use serum-free medium for adherent cells, after the addition of 2 mM glutamine. The formulation contains no albumin, which has been found to be non-essential for cell growth, and even prevents efficient adhesion in some cases. The protein content of BIO-MPM-1 is therefore less than 30mg per liter, and the medium contains no growth factors or hormones other than insulin. The formulation also contains no attachment factor, which in many (but not all) cases must be added for successful use.

Adaptation of Cells

In most cases it is possible to seed the cells that have been removed from freezing medium directly in BIO-MPM-1, when the cell concentration is at least $5x10^5$ cells per $25cm^2$. The cells will begin to grow in BIOMPM-1, and after a few passages the adaptation will be complete. However, in those cases where the cells do not adapt successfully after direct transfer, it will be necessary to perform gradual adaptation (weaning). The cells should be seeded with BIO-MPM-1 containing 5% serum and the serum concentration is then gradually reduced with each passage. The stage at which serum is completely removed is determined in the course of the weaning for each specific case. In order to save time, we recommend parallel experiments with direct adaptation and with weaning. Generally, after the first or second passage, it will be obvious whether direct adaptation has been successful, and if not, only the weaning experiments are continued. As part of these experiments it is also necessary to test for the possible requirement of the addition of fibronectin. After successful adaptation, it is recommended to cryopreserve the cells in Serum-Free Freezing Medium, in order to avoid the necessity of any further adaptation in the future.

Growth of Various Anchorage Dependent Cells in BIO-MPM-1 as Compared with Conventional Serum-Supplemented Medium [1]

	10% FBS			BIO-MPM-1		
Cell	Seeding density/ cm^2	Doubling time (hours)	Maximum density/ cm^2	Additives	Doubling time (hours)	Maximum density/ cm^2
3T3	$5x10^3$	24.0	$3.3x10^5$	Bombesin SBTI[3] Fibronectin	25.2	$3.3x10^5$
A-549	$1x10^4$	26.4	$4.5x10^5$	----	33.0	$2.8 x10^5$
B16-F10	$5x10^3$	30.0	$5.0x10^5$	----	30.0	$5.5 x10^5$
BGM	$1x10^4$	19.2	$4.0x10^5$	Fibronectin	30.5	$3.4 x10^5$
BHK-21	$2.5x10^4$	14.4	$4.5x10^5$	Fibronectin	12.0	$9.0 x10^5$
BS-C-1	$1x10^4$	24.0	$2.8x10^5$	----	28.0	$1.9 x10^5$
CEF	$1.2x10^4$	28.8	----	----	36.3	----
HELA	$5x10^3$	48.0	$6.5x10^5$	Fibronectin	36.0	$6.0 x10^5$
HEp-2	$5x10^3$	57.0	$5.5x10^5$	Fibronectin	30.0	$6.5 x10^5$
MA-10[2]	$2.5x10^4$	18.0	$2.7x10^5$	Fibronectin	16.5	$3.8 x10^5$
VERO	$5x10^3$	16.5	$4.1x10^5$	Fibronectin	18.0	$3.8 x10^5$

[1] MEM + 10% FBS: 3T3, A-549, BHK-21, BS-C-1, VERO.
RPMI-1640 + 10% FBS: B16-F10, BGM, HELA, HEp-2
M-199/F10 (1:2): CEF

[2] Cells do not grow with FBS but with 15% horse serum in RPMI

[3] Soybean trypsin inhibitor

Recommended Amounts of Sodium Bicarbonate and L-Glutamine to be Added in the Preparation of Single Strength Liquid Media (1x) from Concentrated Media (10x)

Product Name	Desired Product (1x) Cat. No.	Prepared from Product (10x) Cat. No.	Quantity Sodium Bicarbonate Solution 7.5% Cat. No. 03-040-1 ml/Liter	Quantity L-Glutamine Solution 200mM Cat. No. 03-020-1 ml/Liter
BIO-MPM-1, Multi Purpose SFM	05-060-1	05-060-5	26.9	10-20

CHO Cells

BIOCHO-1 Serum-Free Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOCHO-1 Serum-Free Medium Base Without L-Glutamine	05-061-1A 05-061-1B	500ml 100ml	2-8ºC 2-8ºC
BIOGRO-CHO Serum-Free Medium Supplement 100X Conc.	05-620-1E 05-620-1F 05-620-1H	50ml 1ml 5ml	-20ºC -20ºC -20ºC

BIOCHO-1 SFM Base is the basic formulation for CHO cells. The solution contains amino acids, vitamins, salts, lipids and trace elements.
This medium is intended for the growth of CHO cells of various kinds: CHO-K1, and transfected cells containing recombinant DNA related to the DHFR gene.

BIOGRO-CHO SFM Supplement contains proteins and other components that require storage at -20°C. This product is a 100-fold concentrate. Preparation of the complete medium is carried out by adding 1% BIOGRO-CHO SFM Supplement to BIOCHO-1 SFM Base, and glutamine is then added.
The complete medium does not contain albumin, growth factors or hormones, other than insulin. Total protein concentration is less than 30mg per liter.
After preparation, the complete medium can be stored for up to 30 days at 2-8°C. Prolonged exposure to light should be avoided.

Adaptation of CHO Cells
In most cases it is possible to seed CHO cells that have been removed from freezing medium directly in the serum-free medium, when the cell concentration is at least $5x10^5$ cells per $25cm^2$. The cells will begin to grow, and after a few passages the adaptation will be complete. However, in those cases where the cells do not adapt successfully after direct transfer, it will be necessary to perform gradual adaptation (weaning). The cells should be seeded with serum-free medium containing 5% serum and the serum concentration is then gradually reduced with each passage. The stage at which serum is completely removed is determined in the course of the weaning for each specific case.
In order to save time, we recommend parallel experiments with direct adaptation and with weaning. Generally, after the first or second transfer, it will be obvious whether direct adaptation has been successful, and if not, only the weaning experiments are continued.
After successful adaptation, it is recommended to cryopreserve the cells in Serum-Free Freezing Medium, in order to avoid the necessity of any further adaptation in the future.

Mononuclear Cells (Immunology)

BIOTARGET™-1 Serum-Free Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOTARGET™-1 Without L-Glutamine	05-080-1A 05-080-1B	500ml 100ml	2-8ºC 2-8ºC

BIOTARGET™-1 has been developed specifically for use with human mononuclear cells (lymphocytes and monocytes) from peripheral blood. In work with these cells and their sub-populations, it is critical to optimize and define the media formulation as well as pH and temperature.

In most cases, up until now, these cells are grown in convential media, supplemented with human serum (A, AB) or foetal bovine serum. However, the use of serum suffers from the following disadvantages:
- The serum may contain non-specific growth factors, which interfere with complete activation in the desired direction.
- The serum may contain inhibitors which will limit activation of the lymphocytes.
- Lot to lot variation is certain.
- Pathogens may be introduced via the serum.
- The evaluation of the antigenic reaction, such as the quantity of the lymphokines generated, and the reaction of the lymphokines to hormones and growth factors are all more accurate in the absence of serum.

Applications for BIOTARGET™-1
The applications for the use of BIOTARGET™-1 are numerous and include:
1. Activation of mononuclear cells with the aid of various mitogens (PHA, CON.A, OKT-3).
2. Activation of mononuclear cells with lymphoid cells (RAJI, PEER, BA, MOLT-4, JURKAT).
3. Production of IL-2 and IL-3 from mononuclear cells.
4. Long-term culture of mononuclear cells after activation.
5. Activation of mononuclear cells with interleukin-2 in order to generate LAK or TIL cells.
6. Activation of mononuclear cells in order to generate natural killer cells (NK).
7. Activation of mononuclear cells in order to generate cytotoxic T cells.
8. Activation of macrophages.
9. Research on the influence of various cytokines on the production of sub-populations of mononuclear cells.
10. Proliferation of the HIV virus.
11. Proliferation of retroviruses in T cells for the purposes of vaccine development
12. Proliferation of retroviruses in T cells for the purposes of vaccine development

Following are several examples of the evaluation protocols by which BIOTARGET™-1 was selected:

1. **Mitogenic Activation of Mononuclear Cells**
   Activation was evaluated with different mitogens such as PHA, CON.A and OKT-3. Proliferation was checked by measurement of the uptake of radioactive thymidine. The mitogens were added in varying concentrations and thymidine uptake was determined over several days, in order to fully evaluate the specific medium formulation.
2. **Activation of Mononuclear Cells**
   with Lymphoid Cells The activation of the mononuclear cells was carried out using lymphoid cells of various kinds, such as: JURKAT, RAJI, MOLT-4, and BA. Varying ratios between the tumor cells and the mononuclear cells were examined, and the proliferation was checked by measurement of the uptake of radioactive thymidine.
3. **Production of Lymphokines by Activated Mononuclear Cells**
   The levels of the lymphokines IL-2 and IL-3 were measured in the culture of the mononuclear cells after activation with various mitogens. IL-2 production was measured with the help of the CTLL-2 cell line. These are cytotoxic T-cells from mice, which grow only in the presence of IL-2 in the culture medium.
4. **Cytotoxicity**
   Mononuclear cells were seeded at a concentration of 10^6 cells per well together with RAJI cells which had been treated with mitomycin C. Varying ratios of the two cell types were examined. At the conclusion of the activation (5-7 days), the lymphocytes were collected, centrifuged, suspended in medium and seeded in microwells in order to measure proliferation and cytotoxicity. RAJI cells were labeled with radioactive chromium (10 pCi in a volume of 0.2 ml), washed three times, suspended at a concentration of 105 cells per ml, and divided into microwells containing the above activated lymphocytes. After 18 hours incubation, the cytolytic activity was evaluated by measuring the radioactive chromium released from the target (RAJI) cells.

Insect Cells

BIOINSECT-1 Serum-Free Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOINSECT-1, With L-Glutamine	05-050-1A	500ml	2-8ºC

BIOINSECT-1 is a serum-free medium optimized for the culture of lepidopteran insect cells. The medium supports both suspension and stationary cultures of Sf-9 cells derived from the pupal ovarian tissue of Spodoptera frugiperda. Sf-9 cells are suitable hosts for the replication of the baculovirus Autographa colifornica nuclear polyhedrosis virus. This virus, isolated from the Alfalfa looper, is used for the recombinant expression of heterologous proteins in the baculovirus expression vector system (BEVS). Insect cells, infected with this virus, display accumulations of the highly expressed protein polyhedrin, within the nuclea (polyhedra). This protein-free medium supports the growth of Sf-9 cells with significantly better results than those obtained using TNM-FH medium (supplemented Grace's) with 10% foetal bovine serum, and production of recombinant beta-galactosidase is also excellent. BIOINSECT has showed excellent performance when cultivating high-V cells.

Weaning Procedure

Transfer cells in the logarithmic phase from the serum-containing medium into 50% (v/v) mixture of serum-supplemented medium and BIOINSECT-1.

Subculture the cells after 3 days and reduce the percentage of the serum-supplemented medium to 40%.

Continue with the subculturing of the cells every 3 days and with each passage reduce the concentration of the serum-supplemented medium by a further 10%. On the sixth passage, the cells will be fully adapted to BIOINSECT-1 serum-free medium.

Maintenance of Sf-9 cells in BIOINSECT-1 serum-free medium:

	Stationary culture	Suspension culture
Inoculation density	6-10 x 10^4 cells/cm^2 2-3 times/week	1.5 x 10^6 cells/ml Every 3-4 days
Subculture	Subculture the cells when the viable cell count reaches 4-5 x 10^5/cm^2, with greater than 90% viability.	Subculture the cells when the viable cell count reaches 3-5 x 10^6/ml, with greater than 95% viability. After 5 days in culture, the cell density reaches 6-8 x 10^6 cells/ml.

The culture may be gently centrifuged when subculturing, in order to remove the toxic by-products in the supernatant.

Cryopreservation

Serum-Free Cell Freezing Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
Serum-Free Cell Freezing Medium PF, ACF	05-065-1A 05-065-1B	500ml 20ml	2-8ºC 2-8ºC

Protein-Free, Animal Component- Free (ACF)

When using serum-free media in mammalian cell culture, it is important to cryopreserve cells also in a medium free of serum. The novel cell freezing medium that has been developed by Biological Industries contains no serum, no proteins and no animal components but rather methylcellulose and DMSO. After freezing and thawing, a very high percentage of viable cells is obtained, and they also show excellent attachment ability as well as growth performance. In fact comparative studies have shown that in most cases higher viabilities and adhesion percentages are obtained in comparison to serum-containing freezing medium. Therefore, the use of this serum-free freezing medium is also recommended for cell culture employing serum-supplemented growth media.

Performance Validation

Serum-free Freezing Medium is a complete, ready to use solution which is designed to protect frozen cells in liquid nitrogen for long-term storage, without any use of protein or other animal components.

1. **Materials and methods**

1.1 **Cell lines**

Various cells grown under serum-free conditions were frozen with serum-free freezing medium and with freezing medium containing serum.

1.2 **Freezing method**

Serum-free Freezing Medium (Cat. no.: 05-065-1) and basal medium containing 10% DMSO and 20% FBS were used as freezing media. The cells were frozen in the appropriate freezing medium in a concentration of 3-5x10^6 per ml. One ml of these cell suspensions was transferred to a plastic ampoule and frozen by decreasing the temperature at a rate of 1-2°C/min. The ampoules were kept in liquid nitrogen until tested.

1.3 **Cell recovery measurements**

When thawing, the frozen ampoules were put in a water bath at 37°C. After dilution with culture medium and centrifugation, the cells were resuspended with either serum-free medium or medium containing serum. Viability of cells was determined by the trypan blue dye exclusion method. Adhesion of cells was determined by counting the attached cells only 6-24 hours after culture of the cells.

2. **Results**

2.1 Freezing of cells in Serum-free Freezing Medium in comparison to freezing medium containing serum:

Table 1: Thawing of cells 24 hours after freezing in liquid nitrogen

	Viability %		Adhesion %	
Cell	Serum-free Freezing Medium	Freezing Medium Containing Serum	Serum-free Freezing Medium	Freezing Medium Containing Serum
3T3	85	83	100	83
BGM	91	83	88	88
VERO	66	71	62	33
HEp-2	75	69	100	92
BSC-1	82	77	22	10

2.2 Long term storage of cells in liquid nitrogen using Serum-free Freezing Medium.

Table 2: Recovery of cells frozen in Serum-free Freezing Medium

	24 hours storage		6 months storage		4 years storage	
Cell	Viability	Adhesion	Viability	Adhesion	Viability	Adhesion
B16-F10	85	100	74	79	72	80
BGM	80	70	61	100	66	92
BHK-21	80	93	64	100	71	95
HELA	87	78	70	90	80	83
HEp-2	90	100	63	100	66	94
MA-10	88	95	81	100	83	91
VERO	90	94	71	68	73	76

3. **Summary**

The Serum-free Freezing Medium supports efficient cryopreservation of various cell lines cultured in serum-free media. After freezing and thawing, a very high percentage of viable cells is obtained, and they also show excellent attachment ability as well as growth performance. In fact, the present study has shown that in most cases higher viabilities and adhesion percentages are obtained in comparison to freezing medium containing serum. Therefore, the use of this Serum-free Freezing Medium is also recommended for cell culture employing serum-supplemented growth media.

Method of use

It is recommended to detach the adherent cells (to be frozen) with crystalline trypsin solution, and neutralization with Soybean Trypsin Inhibitor Solution. After centrifuging, suspend the cells in cold serum-free freezing medium at a concentration of 3-5 million cells per ml. Freeze the cells gradually (1-2°C per minute) and store them in liquid nitrogen. Thawing should be performed at 37°C. Immediately after thawing, suspend the cells in serum-free growth medium at a ratio of at least 1:10. Then centrifuge and suspend at high concentration in growth medium.

Auxiliary Solutions

Cell Dissociation Solution (Non-Enzymatic)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Cell Dissociation Solution (non-enzymatic)	03-071-1B	100ml	2-8°C

Cell Dissociation Solution is a special, non-enzymatic formulation with a proprietary mixture of chelators for gently dislodging adherent cell types from culture vessels. Cell Dissociation Solution helps to maximize the yield of functionally viable cells from these culture vessels. It is a non-enzymatic, protein-free and animal component-free solution. Another major advantage is that cells can be exposed to this solution for longer periods of time without the risk of subjecting them to protein digestive enzymes such as trypsin. However, the solution is not recommended for cells with very adhesive properties. For those cell lines which are difficult to dislodge, Biological Industries has developed a Papain Dissociation Solution.

Features

Contains a proprietary mixture of chelators. Contains no enzymes or proteases.

- Works with serum-free and serum-containing media.
- Reduces the risk of cell damage associated with trypsin.
- Chemically defined.
- Contains no products of animal origin.
- Supplied as a ready-to-use solution.

Papain Dissociation Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Papain Dissociation Solution	03-072-1B	100ml	-20°C

Papain is a nonspecific, endolytic, sulfhydrl protease or protein-cleaving enzyme, known as cysteine-endopeptidase, and is derived and isolated from papaya fruit (i.e. Carica papaya). More specifically, it is isolated from the papaya latex, which is then utilized in a wide variety of applications. Papain is commonly used in cell isolation procedures, where it has proven to be more efficient and less destructive than other proteases on certain tissues such as and including, among others, the dissociation of retinal neurons[1], in the preparation of primary neurons from the visual cortex of postnatal rats[2], and for the isolation of smooth muscle cells[3].

Papain has a wide specificity in that it will degrade most protein substrates more extensively than the pancreatic proteases and has been proven not only to manifest fewer untoward and negative ramifications producing less cell and tissue trauma, but also to be much more effective than other available proteases. Biological Industries' Papain Dissociation Solution is a ready-to-use solution and is one of our non-animal alternatives for trypsin.

Physical Properties and Kinetics

Papain is a cysteine protease hydolase enzyme of the peptidase C1 family derived from the papaya family, Carica papaya and the mountain papaya, Vasconcellea cundinamarcensis. It consists of a single peptide chain with three disulfide bridges and a sulfhydrl group necessary for the activity of the enzyme.

Specificity

Papain is more effective in digesting most protein substrates more extensively and effectively than pancreatic proteases. It further exhibits broad specificity cleaving peptide bonds of such basic amino acids as leucine and glycine. In addition to the aforementioned activity, it also hydrolyzes esters and amides.

[1] Shen J., et al., Japanese Journal of Physiology, 1995
[2] Huettner, J.E. Baughman, R.W., Journal Of Neuroscience, 1986
[3] Kinoshita, K. et.al., American Journal of Physiology, Gastrointestinal and Liver Physiology, 2003 and Driska, S.P. et.al., Journal of Applied Physiology, 1999.

Human Fibronectin Solution, 1mg/ml

Product Name	Catalogue No.	Unit Size	Storage Temp.
Human Fibronectin Solution, 1mg/ml	05-750-1H	1ml	2-8ºC
	05-750-1F	5ml	2-8ºC

Human Fibronectin (hFN) was tested and found suitable matrix for many cell types as well as for stem cells (e.g. mesenchymal stem cells). Biological industries hFN is obtained by affinity purification on gelatine-sepharose from human plasma.

Features

- A complete ready-to-use solution.
- Suitable for various animal cells.
- Performance tested.

Bovine Fibronectin Solution, 1mg/ml

Product Name	Catalogue No.	Unit Size	Storage Temp.
Fibronectin Solution (Bovine), 1mg/ml	03-090-1-01	1ml	2-8ºC
	03-090-1-05	5ml	2-8ºC

Fibronectin is an attachment factor that facilitates the attachment and cytoplasmic spreading of all types of anchorage-dependent cells. Fibronectin is particularly useful for the culture of cells that are not capable of synthesizing their own biomatrix, or when culturing cells in serum-free medium.

Suggested Coating Procedures

The Fibronectin should be added to the growth medium in the growth vessel, which is then placed in an incubator 30-60 minutes before seeding. The recommended concentration of the Fibronectin is 5 micrograms per ml of medium. When the medium is replaced in the days following initial seeding, no further Fibronectin is required.

Crystalline Trypsin Solution & Soybean Trypsin Inhibitor Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Crystalline Trypsin Solution (0.02%) Without Phenol Red	03-047-1A	500ml	-20ºC
	03-047-1B	100ml	-20ºC
Soybean Trypsin Inhibitor 50X Conc., 5mg/ml	03-048-1C	20ml	-20ºC

Crude trypsin is often the subculturing agent of choice for cell dissociation/disaggregation of adherent cells, although the treatment may be cytotoxic if prolonged. Over-trypsinization is a common cause of subculture problems. Regarding the use of crude trypsin, some important facts must be noted:

- Cells must **NEVER** remain in the crude trypsin for longer than 3-5 minutes as they may be seriously damaged in the process (i.e. damage to the intracellular proteins).
- Cells should **NEVER** be left without a fluid layer.

The use of crystalline trypsin, rather than crude trypsin, most often performs better long-term cell growth in serum-free medium formulations. It is specifically formulated to have a gentle nature with much better cell viability, in which the cells are not subject to the vagaries of time and circumstance as when the cruder forms of trypsin are utilized.

Some of the advantages of crystalline trypsin versus the cruder trypsin forms:

1. Crystalline trypsin does not damage cells after prolonged exposure.
2. Crystalline trypsin does not require multiple-change procedures and thus is less labor-intensive.
3. Crystalline trypsin maintains better cell viability and enhances the process of cell passaging.
4. Crystalline trypsin is not as cytotoxic to cells with all the negative ramifications of crude trypsin.
5. Biological Industries' Crystalline Trypsin Solution also contains additives that protect the cell wall, enhancing cell viability.

In a serum-free culture environment, the cells must be separated by rapid centrifugation or by utilizing trypsin inhibitors such as Soybean Trypsin Inhibitor (SBTI). SBTI is a single polypeptide that forms a stable, stoichiometric, enzymically inactive complex with trypsin, thereby reducing the availability of trypsin by somewhat binding chymotrypsin. With Biological Industries' Soybean Trypsin Inhibitor Solution, any excess Crystalline Trypsin Solution may be completely neutralized, thereby avoiding the use of serum for this purpose. The cells may then be re-suspended successfully in a suitable growth medium.

The use of animal-derived components in Biopharmaceutical Manufacturing is experiencing ever-increasing regulatory scrutiny. Therefore, there is the need to develop non-animal source products for cell culture. Trypsin is an essential product for cell culture manipulation. However, it is purified from animal-source materials with one unfortunate notable disadvantage: contamination from variegated sources such as viruses, other potential adventitious agents and other unwanted enzymes.

Ordering Information

Product Name	Catalogue No.	Unit Size	Storage Temp.
Vero Cells			
NutriVero™ VP1, Animal Component-Free Serum-Free Medium for the Monolayer Culture of Vero Cells (NutriVero™ VP1, ACF SFM)	05-066-1A 05-066-1B	500ml 100ml	2-8ºC 2-8ºC
NutriVero™ VP2, Animal Component-Free Serum-Free Medium for the Microcarrier Suspension Culture of Vero Cells (NutriVero™ VP2, ACF SFM)	05-067-1A 05-067-1B	500ml 100ml	2-8ºC 2-8ºC
Hybridoma Cells			
DCCM-1 without L-Glutamine	05-010-1A 05-010-1B	500ml 100ml	2-8ºC 2-8ºC
DCCM-1 10X Conc., Without L-Glutamine without Sodium Bicarbonate	05-010-5B	100ml	2-8ºC
DCCM-2, without L-Glutamine	05-015-1A 05-015-1B	500ml 100ml	2-8ºC 2-8ºC
DCCM-2 10X Conc., Without L-Glutamine, Without Sodium Bicarbonate	05-015-5A 05-015-5B	500ml 100ml	2-8ºC 2-8ºC
Low Protein Media BSA-Free (LPM) Without L-Glutamine	05-040-1A 05-040-1B	500ml 100ml	2-8ºC 2-8ºC
Low Protein Media BSA-Free (LPM) 10X Conc., Without L-Glutamine, Without Sodium Bicarbonate	05-040-5B	100ml	2-8ºC
BIOGRO-1 Serum-Free Media Supplement 50X Conc.	05-600-1B 05-600-1C 05-600-1D 05-600-1T	100ml 20ml 10ml 2ml	-20ºC -20ºC -20ºC -20ºC
BIOGRO-2 Serum-Free Media Supplement 50X Conc.	05-610-1B 05-610-1C 05-610-1D 05-610-1T	100ml 20ml 10ml 2ml	-20ºC -20ºC -20ºC -20ºC
Anchorage-dependant Cells			
BIO-MPM-1,Multi-Purpose SFM Without L-Glutamine	05-060-1A 05-060-1B	500ml 100ml	2-8ºC 2-8ºC

Product Name	Catalogue No.	Unit Size	Storage Temp.
CHO Cells			
BIOCHO-1 SFM Base Without L-Glutamine	05-061-1A 05-061-1B	500ml 100ml	2-8ºC 2-8ºC
BIOGRO-CHO Serum-Free Media Supplement 100X Conc.	05-620-1E 05-620-1F 05-620-1H	50ml 1ml 5ml	-20ºC -20ºC -20ºC
Mononuclear Cells (Immunology)			
BIOTARGET™-1 Without L-Glutamine	05-080-1A 05-080-1B	500ml 100ml	2-8ºC 2-8ºC
Insect Cells			
BIOINSECT-1, With L-Glutamine	05-050-1A	500ml	2-8ºC
Cryopreservation			
Serum-Free Cell Freezing Medium	05-065-1A 05-065-1C	500ml 20ml	2-8ºC 2-8ºC
Auxiliary Solutions			
Crystalline Trypsin Solution (0.02%) Without Phenol Red	03-047-1A 03-047-1B	500ml 100ml	-20ºC -20ºC
Soybean Trypsin Inhibitor 50X Conc., 5mg/ml	03-048-1C	20ml	-20ºC
Cell Dissociation Solution (non-enzymatic)	03-071-1B	100ml	2-8ºC
Papain Dissociation Solution	03-072-1B	100ml	-20ºC
Bovine Fibronectin Solution, 1mg/ml	03-090-1-01 03-090-1-05	1ml 5ml	2-8ºC 2-8ºC
Human Fibronectin Solution, 1mg/ml	05-750-1H 05-750-1F	1ml 5ml	2-8ºC 2-8ºC

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