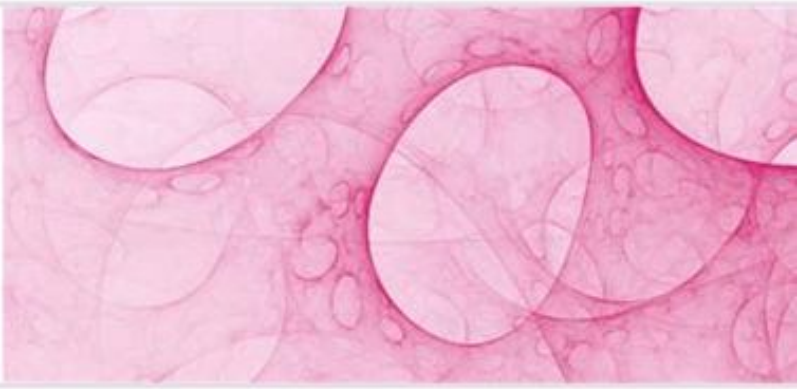
TRYPSIN AND TRYPSIN SUBSTITUTES

CELL DISSOCIATION PRODUCTS



Most cell cultures grow as a single thickness cell layer or sheet attached to a substrate known as a monolayer. When subculturing adherent cells, these intercellular and cell-to-substrate links or connections must be gently dissociated. Proteolytic enzymes, such as trypsin (i.e. a serine peptidase), breaks or gently separates these bonds by creating a single-cell suspension from which new subcultures are realized. Trypsin solutions are widely utilized as cell dissociation reagents for continuous cell culture of adherent growing cells. Trypsin proteolysis or trypsinization is a process in which proteins have been digested or treated with trypsin and are thus said to be trypsinized. Biological Industries' Trypsin is designed to gently dissociate cells from almost any support substrates, as well as from each other, in order to actualize cell manipulation techniques, and for other studies that require intact cell-surface proteins. Trypsin is available in a varied array of formulations with or without EDTA. EDTA is a chelator that binds calcium and magnesium ions that may otherwise inhibit the trypsin activity, which then hydrolyzes and gains access to the intercellular bonds (cell-cell and/ or cell-substrate bonds).

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.

Non-Enzymatic Cell Dissociation Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Non-Enzymatic Cell Dissociation Solution	03-071-1B	100ml	2-8ºC

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	-20ºC

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

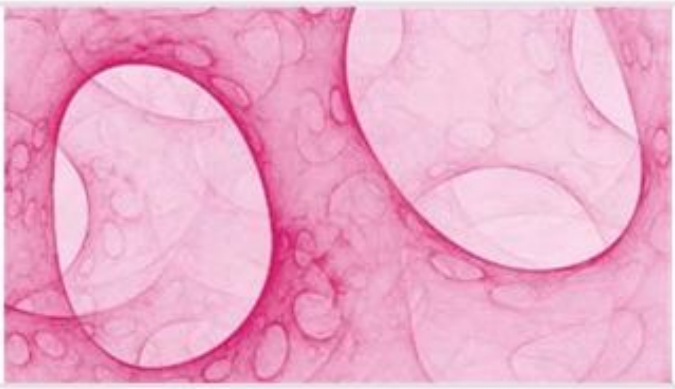
Papain is more effective in digesting most protein substrates more extensively and effectively than pancreatic proteases. It further exhibits broad specificity cleaving peptide bonds of such basic amino acids as leucine and glycine. In addition to the aforementioned activity, it also hydrolyzes esters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

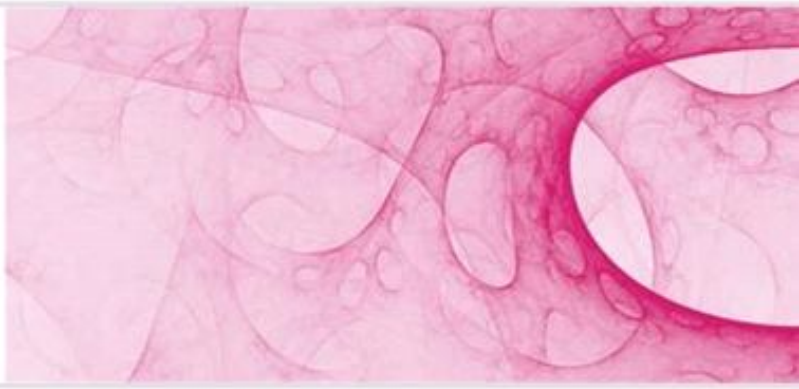
(1) Shen J., et al., Japanese Journal of Physiology,1995
(2) Huettner, J.E. Baughman, R.W., Journal Of Neuroscience, 1986
(3) Kinoshita, K. et.al., American Journal of Physiology, Gastrointestinal and Liver Physiology, 2003 and Driska,S.P. et.al., Journal of Applied Physiology, 1999.

Accutase Solution, primary human cell culture tested

Product Name	Catalogue No.	Unit Size	Storage Temp.
Accutase Solution, primary human cell culture tested	03-073-1B	100ml	-20ºC

Accutase is an alternative cell detachment solution to trypsin and can also be used for tissue dissociation. It is a ready to use solution and was developed for very gentle and effective detachment of adherent cells. The well balanced combination of protease and collagenolytic activities ensures that surface proteins and epitopes stay entirely intact. This makes it perfectly suited for applications, which require unchanged surface conditions.





Product Name	Trypsin Concentration	Catalogue No.	Unit Size	Storage Temp.
Trypsin Solution A With Calcium and Magnesium Without Phenol Red	0.25%	03-045-1B	100ml	2-8ºC
Trypsin Solution B Without Calcium and Magnesium Without Phenol Red	0.25%	03-046-1A	500ml	-20ºC
		03-046-1B	100ml	-20ºC
Trypsin Solution B Without Calcium and Magnesium Without Phenol Red 10x Concentrate	2.50%	03-046-5A	500ml	-20ºC
		03-046-5B	100ml	-20ºC
Crystalline Trypsin Solution Without Phenol Red	0.02%	03-047-1A	500ml	-20ºC
		03-047-1B	100ml	-20ºC
Soybean Trypsin Inhibitor 50x Conc., 5mg/Ml		03-048-1C	20ml	-20ºC
Trypsin EDTA Solution A EDTA (0.02%) With Phenol Red	0.25%	03-050-1A	500ml	-20ºC
		03-050-1B	100ml	-20ºC
Trypsin EDTA , EDTA 0.2% , 10X Conc.*	0.50%	03-051-5B	100ml	-20ºC
		03-051-5C	20ml	-20ºC
Trypsin EDTA Solution B EDTA (0.05%) With Phenol Red	0.25%	03-052-1A	500ml	-20ºC
		03-052-1B	100ml	-20ºC
Trypsin EDTA Solution C EDTA (0.02%) With Phenol Red	0.05%	03-053-1A	500ml	-20ºC
		03-053-1B	100ml	-20ºC
Trypsin EDTA Solution C EDTA (0.02%) Without Phenol Red	0.05%	03-054-1A	500ml	-20ºC
		03-054-1B	100ml	-20ºC
Non-Enzymatic Cell Dissociation Solution		03-071-1B	100ml	2-8ºC
Papain Dissociation Solution		03-072-1B	100ml	-20ºC
Accutase Solution, primary human cell culture tested		03-073-1B	100ml	-20ºC

* Cytogenetics> information


BIOLOGICAL INDUSTRIES
KIBBUTZ BEIT HAEMEK ISRAEL
STERILE CE
TRYPSIN INHIBITOR
SOLUTION (From soybean)
CONCENTRATE x 50
Store at -20°C
Cat. 03-048-1B
Exp. 10/2009
Vol. 100ml
Lot: 818682
For In Vitro Diagnostic Use
www.biolind.com
CRYSTALLINE TRYPSIN
SOLUTION
200 mg/l
CELL DISSOCIATION
SOLUTION
Non-Enzymatic
Animal component free
Cat. 03-071-1B
Store: +2 to 8°C.
Exp: 05/2011
Vol. 100ml
Lot: 919550