Product Profile

Product Name:	*Non-Enzymatic Cell Dissociation Solution in Dulbecco's Phosphate Buffered Saline without Calcium and Magnesium*
Catalog Number	*03-071-1*
Unit Size Availability:	(A)500ml;(B)100ml;(C)20ml
Formulation:	Liquid Solution
Defined Storage Conditions:	2-8°C
Stability: (Under Defined Handling & Storage Conditions)	Please Refer To Product Label

Important Note! Please read the ***MSDS*** and ***Product Profile*** carefully in their entirety ***before*** using this material for possible safety precautions and potential hazards.

Product Description:

Most cell cultures grow as a single thickness or sheet attached to a substrate known as a monolayer. When culturing adherent cells, these intercellular and cell-to-substrate links or connections must be gently dissociated. Normal attachment, growth and development of many cell types are dependent on attachment factors and extracellular matrix components. Attachment factors are structural proteins that have adherent-like capabilities and increase cell-substrate interactions in a culture dependent attachment milieu. Well-known proteolytic enzymes such as Trypsin break or gently separate these bonds by creating a single-cell suspension from which new subcultures are realized. These serine peptidases are widely utilized as cell dissociation reagents for continuous cell culture or adherent growing cells. However, Trypsin, although an essential product for cell culture manipulation, and purified from animal source raw materials like other animal-derived components, is experiencing ever-increasing regulatory scrutiny especially in Biopharmaceutical Manufacturing. Therefore, there was a need to develop protein-free, non-animal source Trypsin alternative products for cell culture due to the potential for untoward contamination from variegated sources such as viruses, other adventitious agents and/or unwanted enzymes.

Tissue dissociation, primary cell isolation and cell harvesting are some of the major applications for the utilization of enzymes not only in cell culture in particular but also in cell biology research as a whole. In spite of their widespread use, their mechanisms of action (MOA's) are not well understood and therefore, the choice of one enzyme or technique over another is often empirical and arbitrary. Maximizing the yield of functionally-viable, dissociated cells are often dependent upon, but not limited to the following parameters:

- Type of Tissue
- Species
- Age
- Genetics
- Dissociation Medium Utilized
- Crude Enzyme Impurities
- Enzyme Concentrations Utilized
- Temperature
- Incubation Times

Non-Enzymatic Cell Dissociation Solutions (CDS) are often employed when it is necessary to harvest cells by gently dislodging adherent cell types from culture vessels especially when non-protein and animal-component free materials are the order of the day. This proprietary mixture of chelators is a good alternative to protein digesting animal-source proteins, other proteolytic enzymes and proteases such as Collagenases, Pronases and varied arrays of Trypsin formulations also used for Cell Dissociation/Disaggregation to actualize cell manipulation techniques. The presence of certain proteases may interfere and/or modify cell membranes and cellular proteins causing untoward manifestations in physiological or immunological assays.

This non-enzymatic CDS helps not only to maximize the yield of functionally viable cells from culture vessels without the often untoward and cytotoxic effects of enzymes, but also with the advantage that the cells may be exposed for longer periods of time without the negative ramifications of protein digestive enzymes and over-trypsinization that is a common cause of subculture problems; cells dissociate easily from plastic or glass culture vessels and from each other. Another prominent advantage is that it is less labor-intensive, not subject to such lot/batch variability or the worry of other traces of interfering enzymes that might be present. However, this solution is not recommended for cell lines with very adhesive properties. Cell viability is also dependent upon the laboratory technique employed and in general, how the cells are handled and under what conditions they are cultured.

Some Predominant Characteristics of the Non-Enzymatic Cell Dissociation Solution (CDS) include:

- Ready-To-Use, Chemically Defined, Non-Enzymatic Proprietary Mixture of Chelators
- Non-Animal Source Alternative to Trypsin without its Untoward Cytotoxicity
- Effective with Serum-Free or with Serum-Containing Medium
- Meets USP and EPTesting Specifications
- Suitable for Cell-Culture & Molecular Biology Applications
- Long-Storage When Handled and Stored Properly Under Defined Conditions

Storage & Stability:
This product should be stored under specified conditions (2-8°C) and used within the time frame specified on the label. Do ***not*** use after the expiration date. Deterioration of liquid media may be recognized by any of the following characteristics, among others including: (a). color change, (b). granulation/ clumping, (c). insolubility, (d). and/or decrease in expected performance parameters. Any material described above should not be used and therefore discarded.

Instructions/Procedure:*

1) Pre-warm the Non-Enzymatic Cell Dissociation Solution to 37°C
2) Drain and Discard the medium from the culture vessel without drying out the monolayer
3) Rinse the monolayer with approximately 2.0ml of Dulbecco's Phosphate Buffered Saline (DPBS) ***without*** Calcium and Magnesium or the Non-Enzymatic Cell Dissociation Solution.
4) Add the Non-Enzymatic Cell Dissociation solution to the culture vessel (i.e., ~1.5ml/25ml Tissue Culture Flask) as required and gently swirl the vessel to completely bathe the monolayer.
5) Incubate cells at 37°C by periodically observing the cells under a microscope until they begin to round up. Tapping the side of the vessel will facilitate the removal of more adherent cell lines.
6) After detachment, disperse cells into suspension by pipetting repeatedly.
7) Centrifuge the Cells at 1000 rpms for 2-5 minutes. Remove as much of the Non-Enzymatic Cell Dissociation Solution as possible and re-suspend the pellet on an appropriate medium.

Please Note:

It is important to maintain cells at incubator temperature (i.e., 37°C for Mammalian Cells) as much as possible. The amount of Non-Enzymatic Cell Dissociation Solution utilized and the length of time needed to dislodge cells is often not only directly correlated or dependent upon the cell line but also the medium utilized. When working with Serum-Free Medium (SFM), it is highly recommended to bathe the monolayer with 1mM EDTA in Dulbecco's Phosphate Buffered Saline (DPBS) without Calcium and Magnesium ***before*** the dissociation.

As the selection of a nutrient medium or supplementation thereof is strongly influenced, among others, by many factors, of note are three major considerations:

- ◊ Cell Type
- ◊ Type of Culture(e.g., Clonal, Monolayer, Suspension)
- ◊ Degree of Chemical Definition

It is recommended to review the extensive literature concerning cell-culture media and its supplementation and the physiological parameters required for each specific cell-line as per their essential requirements.

Quality Control

Test	Specifications
Appearance/Description	Clear Solution
Cell-Culture:	Pass
Cell-Line:	Vero
Osmolality:	280-300mOsm/kg
pH:	7.25-7.45
Sterility:	Sterile

Auxiliary Products

Product Name	Catalog Number	Storage Temperature
Dulbecco's Phosphate Buffered Saline(DPBS) without Calcium and Magnesium	02-023-1	Room Temperature(15-30°)
L-Glutamine Solution 29.2mg/ml in Saline	03-020-1	-20°C
L-Alanyl-L-Glutamine Solution(A Dipeptide Substitute)	03-022-1	-20°C
Penicillin-Streptomycin Solution,10,000 units/ml Penicillin G Sodium Salt,10mg/ml Streptomycin	03-031-1	-20°C
Trypsin Solution B (0.25%),Without Calcium and Magnesium, Without Phenol Red	03-046-1	-20°C
Crystalline Trypsin Solution (0.2%) Without Phenol red	03-047-1	-20°C
Soybean Trypsin Inhibitor 50X Conc.,.5mg/ml	03-0148-1	-20°C
Sterile Culture-Grade Water	03-055-1	Room Temperature(15-30°)
Papain Cell Dissociation Solution	03-072-1	-20°C
Serum-Free Cell Freezing Medium	05-065-1	2-8°C
Note: For a list of other Antibiotics, Serum, Reagents and Supplements, please refer to our Product Catalog, Product Profiles, Product Guides and Internet Site.		

References:

1) Current Edition Merck Index
2) Biological Industries (BI)Specifications
3) Current Edition USP/E Ph
4) Martindale The Extra Pharmacopeia,28th Edition, Royal Pharmaceutical Society: London, England