Technical Data Sheet Date: 07/01/2013

Product Code: PM-T1717
Description: Trypsin EDTA 1X Lyophilised w/ Sodium Chloride

Tryspin is the most commonly used enzyme for cell harvesting in Tissue Culture. Trypsin is an animal derived product. The trypsinis derived from porcine pancreatic glands. This avoids the risk of contamination with Bovine Spongiform Encephalopathy (BSE). All glands are derived from animals which were declared healthy at the time of slaughter. In the unlikely event that the glands were contaminated with a virus, even our trypsin undergoes a high acid/high temperature process to inactivate any that may be present.
They are also free of ammonium sulphate.

Storage: +2 to 8° C
Shelf life: 36 months
Composition: Available on request

Recommended Use:
Products supplied by Biosera are for cell culture/in Vitro Laboratory use only, not for drug human or veterinary use.

Applications:
Trypsin is a porcine pancreas derived enzyme that is commonly used for the dissociation and disaggregation of anchorage-dependent mammalian cells and tissues. The concentration of trypsin necessary to dislodge cells from their substrate is deoendent primarily on the cell type and the age of the culture. Trypsin 1X solutions can range from 0.0025% to 0.5%. The reasons for the range of concentrations are as follows:

Differences in trypsin activity or potency
Differences in incubation times
Different cell lines

EDTA, a chelating agent, enhance the enzymatic activity by removing calcium and magnesium ions. These ions obscure the peptide bonds on which trypsin acts as well as enhancing cell to cell adhesion

Utilisation
Supplements as a HBSS solution w/o Calcium w/o Magnesium could be added but the storage conditions will be modified and the shelf life too.

To incubate cells in trypsin's solution that is too concentrate or a time too long will damage the wall of the cells and kill them. If you have doubt about the concentration that should be used for your cells, use a low concentration.

Preparation of the trypsin solution

1. Dilute the trypsin in distilled water
2. While stirring, adjust the pH with 1 N NaOH 0.1-0.3 pH units below the desired pH since it may rise during filtration.
3. Sterilize immediately by filtration using a membrane with a porosity of 0.22 microns.
4. Aseptically dispense medium into sterile container.

Preparation of the trypsinization

Aspirate the spent medium from the culture vessel and discard.

1. Rinse the monolayer with either a small amount of trypsin solution or a calcium and magnesium-free salt solution (as listed below), aspirate, and discard.
2. Dulbecco's Phosphate Buffered Saline (DPBS) catalog N° LM-S2041
3. Hank's Balanced Salt Solution (HBSS) catalog N° LM-S2039
4. Add enough trypsin solution, prewarmed in a 37°C water bath, to completely cover the cell monolayer.
5. Incubate the flask at 37°C, or for more sensitive cultures, at room temperature or 2 to 8°C.
6. When the trypsinization process is complete, cells will appear rounded upon microscopic examination and the solution in the flask will appear cloudy. Check the flask often to avoid overexposure which can damage the cells.
7. The trypsin should be neutralized either with serum containing medium or trypsin inhibitor. Gently centrifuge the cell suspension and discard the trypsin-containing supernatant.
8. Resuspend the cell pellet with fresh medium and count or culture as desired.

Indications of deterioration:

Dry powder reagents should be free flowing. Do not use if powder caked. Prepared medium should be cleared of particulates and flocculent material. Do not use if liquid medium is cloudy or contains precipitate. Other evidence of deterioration may include colour change or degradation of physical or performance characteristics.