

Technical Information

Trypsin-EDTA Solution 10X

2.5% Trypsin (1:250), 0.2% EDTA in 0.85% normal saline

Product Code: TCL1070

Application:-

Trypsin Molecular Weight: 23.4kDa

CAS No: 9002-07-07

EC No: 3.4.21.4

Trypsin is a serine protease derived from porcine pancreas. It is a single chain polypeptide of 223 amino acid residue with substrate specificity based on positively charged Lysine and Arginine side chains. Trypsin predominantly cleaves peptide chains at the carboxyl sides of Lysine and Arginine, except when either is followed by Proline. It is most commonly used for dissociation and disaggregation of adherent cells.

Ethylenediaminetetraacetic acid (EDTA), a chelating agent is often added to enhance enzymatic activity of trypsin solution. EDTA acts by neutralizing calcium and magnesium ions that enhance cell to cell adhesion.

TCL1070 is 2.5% Trypsin, 0.2% EDTA in 0.85% normal saline.

Activity:

One BAEE unit will produce a Δ A253nm of 0.001 per minute with BAEE as substrate at pH 7.6 at 25°C in a reaction volume of 3.2ml (1cm light path).

One TAME unit hydrolyzes 1 μ mole of p-toluenesulfonyl- L-arginine methyl ester (TAME) per minute at 25°C, pH 8.2, in the presence of 0.001M calcium ion.

One USP trypsin unit is the activity causing a change in absorbance of 0.003 per minute under the conditions specified.

Activity Conversion: 1 TAME unit = 19.2 USP or NF units = 57.5 BAEE Units

Methodology

Dilution of 10X solution to 1X solution

1. Thaw frozen Trypsin / Trypsin-EDTA solution in 37°C water bath or overnight at 2-8°C.
2. Aseptically transfer 100ml of 10X Trypsin / Trypsin-EDTA solution to a sterile 1 litre container.
3. Add 800ml sterile calcium magnesium free balanced salt solution. Following salt solutions can be used:

TL2006 - Dulbecco's Phosphate Buffered Saline

TL2034 - Hank's Balanced Salt Solution (With Phenol red)

TL2098 - Hank's Balanced Salt Solution (Without Phenol red)

4. Mix well. Aspirate small quantity of the solution and take pH. If necessary, adjust the pH to 7.0 - 7.6 with sterile 1N NaOH or 1N HCl.
5. Make up the volume to 1 litre.
6. Filter sterilize the solution into appropriate containers.

Dissociation of cells from culture vessel

1. Remove the spent medium from the culture vessel by aspiration.
2. Wash the monolayer by adding balanced salt solution without calcium and magnesium to the side of the flask opposite the cells.
3. Rinse the cell sheet by rocking the flask for 1 to 2 minutes and discard the wash solution.
4. Add Trypsin or Trypsin EDTA solution to the side of the flask opposite the cells. The volume should be sufficient enough to completely cover the monolayer of the cells.
5. Rock the flask to ensure that the dissociation solution covers the cell sheet.
6. Incubate the flask at 37°C for 2 to 3 minutes. Monitor the process by observing the flask under inverted microscope. When dissociation is complete, the cells will be in suspension and appear rounded. In addition to rocking gently, flasks of cell lines that are characteristically difficult to remove from substratum may be tapped to expedite removal.

Note:- The exact time needed to dissociate cells will vary according to the cell line. The dissociation process should be monitored closely to avoid cell damage.

7. Once the cell dissociation is complete add serum containing complete medium to the flask to inhibit the tryptic activity which may further damage the cells.

8. Disperse the cells into a single cell suspension by pipetting repeatedly.

9. Count and subculture the cells.

Note:-

1. Concentration of Trypsin or Trypsin EDTA solution used for dissociation should be determined empirically for individual cell lines.

2. Time required for dissociation of cells from surface depends on cell type, cell density, potency of trypsin, serum concentration in growth medium and time since last subculture.

3. For serum free media, use Soybean Trypsin inhibitor (TCL1068) 1:1 to neutralize the action of trypsin.

Quality control

Appearance

Colorless, clear solution.

pH

7.00 -7.60

Osmolality in mOsm/Kg H₂O

270.00 -310.00

Sterility

No bacterial or fungal growth is observed after 14 days of incubation, as per USP specification.

Cultural Response

Cell Dissociation Test.

Endotoxin Content

NMT 5EU/ml

Storage and Shelf Life

Shelf life of the product is 24 months.

Store the product at -20°C.

Use before expiry date given on the product label.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conforms solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate.
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