UAB "LT Biotech"

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Trypsin-EDTA (0.5 %) in DPBS (10x) LTDe03

General Information

Trypsin-EDTA solutions are used to detach adherent cells from culture surfaces. They are composed of natural porcine pancreas-derived trypsin and EDTA. The concentration of trypsin necessary to dislodge cells from their substrate is dependent primarily on the cell type and the age of the culture. Various formulations should be tested to determine the best product for a specific application.

Appearance	Clear frozen liquid
Storage and shelf life	Store at ≤-15°C. Avoid repeated freeze-thaw cycles. Preparation of aliquots recommended. Once opened, store at 4°C and use within 2-4 weeks.
Shipping conditions	Frozen (Dry ice)
Thawing	+37°C water bath or overnight at +2°C to +8°C. Swirl gently to homogenize.

Formulation

Components	**mg/l**
EDTA 4Na	2200
KCl	200
KH_2PO_4	200
NaCl	8000
Na_2HPO_4	1150
Trypsin	5000

Instructions for Use

Prepare 1x solutions from 10x concentrates

To prepare an acceptable final 1x solution, perform the following procedure under aseptic conditions.

1. The product can either be thawed in a +37°C water bath or overnight at +2°C to +8°C.
2. Aseptically dilute 100 ml of 10x concentrate with approximately 850 ml of a sterile Ca^{2+} and Mg^{2+}-free salt solution (see related products). Mix completely.
3. If necessary, adjust the pH as necessary with 1 N HCl or 1 N NaOH to pH 7.2 – 7.8.
4. Adjust the final volume with the sterile Ca^{2+} and Mg^{2+}-free salt solution.
5. Dispense the solution into sterile containers. Cap the bottles tightly with sterile closures and store at ≤-15°C.

Detachment of adherent cells using Trypsin-EDTA

This entire procedure should be done in a laminar flow hood using proper aseptic technique.

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2. Carefully aspirate all of the media from the cell culture flask.

3. Rinse cells with Ca^{2+} and Mg^{2+}-free salt solution (see related products), aspirate, and discard.

4. Prewarm the 1x trypsin solution in a +37°C water bath. Add enough 1x trypsin solution to completely cover the cells.

5. Incubate the flask at +37°C, or for more sensitive cultures, at room temperature or +2°C 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. Trypsin can cause cellular damage and time of exposure should be kept to a minimum.

   The time required to detach cells from the culture surface is dependent on the cell type, the age of the culture, population density, serum concentration in the growth medium and time since last subculture.

7. Neutralize trypsin either with serum containing medium or trypsin inhibitor. Gently centrifuge the cell suspen- sion and discard the trypsin-containing supernatant.

8. Resuspend the cell pellet with fresh medium and count or culture as desired.

Precautions and Disclaimer

This product is for research use only.