ClearLine	Technical data sheet Media Powder	Ref : 509227

Trypsin EDTA 1X Lyophilised

w/ Sodium Chloride

Storage conditions: -20°C

Shelf life: 36 months

Composition: Displayed on website and in catalogue; also available on request.

pH: 4,5 ± 0,5

Osmolality: 270 ± 27 mOsm/kg

Endotoxin: < 1 EU/ml

Recommended use:
- Respect storage conditions of the product
- Do not use the product after its expiry date
- Store the product in a dry area
- Wear clothes adapted to the manipulation of the product to avoid contamination (e.g. : gloves, mask, hygiene cap, overall…)
- Protect the product from any form of humidity
- Use, in one time, after opening, the entire quantity of product of the container, without making a concentrated solution (to avoid the formation of precipitates). If it is not possible, close the container immediately after sampling the quantity of powder required.
- Supplements can be added prior to sterile filtration of the medium or aseptically introduced to sterile medium (respect the final concentration of the media). The nature of the supplements may affect storage conditions and shelf life of the medium.

The product is intended to be used in vitro for research or further manufacturing only and not for use as an Active Pharmaceutical Ingredient or food or animal feed.

Application:
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 sensitive cells from their substrate, is 0.025% to 0.5%.
EDTA, a chelating agent, enhances 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

Uses:
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.

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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 according concentration required
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

1. Aspirate the spent medium from the culture vessel and discard.
2. 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.
   Dulbecco's Phosphate Buffered Saline (DPBS) CAT N° : L0615
   Hank's Balanced Salt Solution (HBSS) CAT N° : L0611
3. Add enough trypsin solution, prewarmed in a 37°C water bath, to completely cover the cell monolayer.
4. Incubate the flask at 37°C, or for more sensitive cultures, at room temperature or 2 to 8°C.
5. 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.
6. The trypsin should be neutralized either with serum containing medium or trypsin inhibitor. Gently centrifuge the cell suspension and discard the trypsin-containing supernatant.
7. Resuspend the cell pellet with fresh medium and count or culture as desired.

Signs 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.

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