ClearLine	Technical data sheet Media Liquid	Ref : 509601

Trypsin – EDTA in PBS 10X

w/o Calcium w/o Magnesium w/o Phenol Red

Storage -20°C
Repeated freezing and thawing will reduce enzymatic activity and should be avoided.

Shelf life: 24 months

Composition: displayed on website; also available on request

Colour: colourless solution

pH: 7.0 ± 0.3

Osmolality: 450 ± 10% mOsm/kg

Endotoxin: Not applicable

Sterility tests:
- Bacteria in aerobic and anaerobic conditions
- Fungi and yeasts

Cell Growth test: Not applicable

Other tests:
Activity test: Cells detachment test with the L929 cell line

Recommended use:
- Respect storage conditions of the product
- Do not use the product after its expiry date
- Store product in an area protected from light
- Manipulate the product in aseptic conditions (e.g. : under laminar air flow)
- Wear clothes adapted to the manipulation of the product to avoid contamination (e.g. : gloves, mask, hygiene cap, overall…)
- In order to preserve all product qualities, it is recommended to thaw out the flask, to aliquote, then to re-freeze the produced flasks rather than to thaw out and re-freeze the flask at each use.
- It is recommended to use the product immediately after its thaw out.

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 cells from their substrate is dependent primarily on the cell type and the age of the culture. Trypsin 1X solutions can range from 0.025% to 0.5%. The reasons for the range of concentrations are as follows:

- Differences in trypsin activity or potency,
- Different incubation times,
- Different cell lines.

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Uses:
Cell exposure to trypsin solutions should be as brief as possible, as trypsin can be harmful to the membrane proteins of susceptible cells and can also be taken up by the cells via pinocytosis. Serum helps to reduce these effects because it contains both proteins that inhibit tryptic activity and factors that assist in repairing any enzymatic damage done to the cells.
In serum-free conditions, soybean trypsin inhibitor and refrigerated temperatures can help to reduce these undesirable effects.

Methods for use:

1. Frozen products can either be thawed in a 37°C water bath or overnight at to 2 to 8°C.
2. Aspirate the spent medium from the culture vessel and discard.
3. Rinse the monolayer with either a small amount of trypsin or a calcium and magnesium-free salt solution (as listed above), aspirate, and discard.
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.

Signs of Deterioration:
Trypsin solutions should be clear of particulates and flocculent material.
Do not use if solution is cloudy or contains precipitate.
Other evidence of deterioration may include degradation of physical or performance characteristics.

Remarks:
Dilution Instructions for 10X Solutions:

1. Frozen products can either be thawed in a 37°C water bath or overnight at 2 to 8°C.
2. Aseptically transfer 100 ml of 10X trypsin to a sterile one liter container.
3. Add 800 ml of a sterile calcium and magnesium-free salt solution (as listed below) to the container.
   Dulbecco's Phosphate Buffered Saline (DPBS) catalog N° L0615
   Hank's Balanced Salt Solution (HBSS) catalog N° L0611
4. Mix well for several minutes.
5. Determine the pH of a small sample. If necessary, adjust the pH to 7.2-7.8 with 1N HCl or 1N NaOH.
6. Bring the final volume up to 1000 ml with the sterile salt solution and dispense into smaller volumes.

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