ClearLine	Technical data sheet Media Liquid	Ref : 509135

Trypsin 0.25% - EDTA 0.02% in HBSS

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

Storage -20°C

Shelf life: 24 months

Composition: Displayed on website; also available on request

Colour: Orange - red, clear solution

pH: 7.3 ± 0.3

Osmolality: 300 mOsm/kg ± 10 %

Endotoxin: Not applicable

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

Cell Growth test: No cell growth test, but activity test with adherent cells

Other tests: Not applicable

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 non sensitive cells from their substrate, is 0.25%. 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.

Uses:

DOMINIQUE DUTSCHER SAS

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The Trypsin 0.25% EDTA 0.02% in HBSS w/o Calcium w/o Magnesium w/ Phenol Red is a ready to use solution.

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 solution or a calcium and magnesium-free salt solution (as listed below), aspirate, and discard.
   Dulbecco's Phosphate Buffered Saline (DPBS) ref. N° L0615
   Hank's Balanced Salt Solution (HBSS) ref. N° L0611
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:
Medium should be clear and free of particulate and flocculent material.
Do not use if medium is cloudy or contains precipitate.

Remarks:

Storage / Stability:
This product does contain phenol red. The product is shipped on dry ice and there could be significant CO_2 buildup in the package. This CO_2 may enter the solution and lower the pH slightly, giving an orange (around pH 6.5) vs. pinkish (around 7.3) color. The solution, if orange (acidic) should still be good to us as is, or sodium hydroxide may be added to adjust the pH.

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