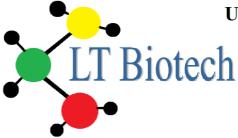
UAB "LT Biotech"



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AccuTOP

Product Description

AccuTOP is a cell dissociation solution containing proteolytic, collagenolytic and DNase enzymes. It is a ready to use nonmammalian, non-bacterial replacement for all applications of trypsin and collagenase in tissue dissociation, cell counting, and dissolving cell clumps such as spheroids. AccuTOP is useful for increasing the accuracy of cell count. Since AccuTOP like Accutase does not affect the surface epitopes of the cells it is therefore ideal for FACS Analysis where even small changes in the cell's properties may have great impact on theresults.

Applications

- Flow Cytometry Analysis
- Cell counting
- Separation of cell aggregates
- Dissociation of cell pellets
- Suitable for suspension cells such as Hybridoma, CHO, BHK and Sf9

Product Specifications

| рН | 6.8 - 7.8 |
|-----------------|--|
| Osmolality | 270 – 320 mOsm/kg |
| Appearance | Clear, pale red to orange solution |
| Sterility Tests | Bacteria, fungi and yeast |
| Activity Test | Cell detachment and enzyme activity assay |
| Concentration | 1x (ready-to-use) |
| Origin | Crustacea |
| Storage | \leq -15°C. Once defrosted, store at +2 to +8°C. After thawing, the AccuTOP solution may be stored for up to 4 weeks at +2 to +8°C. Do not store at roomtemperature. |

Composition

1× AccuTOP enzymes in Dulbecco's PBS without Ca and Mg (0.2 g/l KCl, 0.2 g/l KH₂PO₄, 8.0 g/l NaCl, and 1.15 g/l

 Na_2HPO_4) containing 0.5 mM EDTA · 4 Na.

Protocol for Use

Increasing Reproducibility of Cell Counting

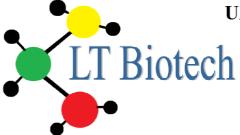
The AccuTOP solution is supplied as a sterile, ready-to-use, frozen liquid. Use aseptic techniques when handling this product.

- 1. Frozen products can either be thawed at room temperature or overnight at +2 to+8 $^\circ C$ (Note:
 - Do not thaw the product in a + 37°C water bath).
- 2. Harvest a representative sample of clumped cells, 0.5 ml or 1.0 ml, and place in an appropriate tube.
- 3. Add an equal volume of AccuTOP to the sample of cells, and incubate for 5 to 10 minutes at room temperature.
- 4. Count the cells by your routine procedure (note that the cells have been diluted an extra 2 fold).

AccuTOP is gentle enough that an aliquot of it can be added to an aliquot of cells and allowed to set for a period of time without damaging the cells.

Dissociation of Primary Tissue

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- 1. Transfer the tissue to a cell culture dish containing fresh, sterile DPBS and rinse.
- 2. Transfer the tissue to a second dish and dissect off unwanted tissue (for example fat or necrotic material).
- 3. Cut the tissue into small pieces by using a scalpel and forceps.
- 4. Transfer the tissue pieces into a 15 or 50 ml sterile centrifuge tube containing fresh, sterile DPBS.
- 5. Allow the tissue pieces to settle and afterwards remove the supernatant. Repeat this wash step two times.
- 6. Transfer the tissue pieces into a new cell culture dish. Add adequate amount of AccuTOP to cover tissue pieces.
- 7. Incubate the sample on a platform rocker for 5 60 minutes at room temperature. The dissociated cells from tissue will "smear" on the bottom of cell culture dish.
- 8. To increase dissociation of cells, gently agitate the sample by pipetting several times.
- 9. After dissociation transfer the cells into a sterile tube and centrifuge at 300 xg.
- 10. Carefully remove supernatant and re-suspend the cell pellet in the appropriate cell culture media. Seed the cells in a suitable cell culture dish. Replace media after 48 hours.

Precautions and Disclaimer

This product is for research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.