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## Accumax, Cell Counting Solution

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### Product Description

- Catalog number:** UPN68091, 100ml
- Name:** **Accumax**, Cell Desegregating Solution for Cell Counting
- Formulation:** 1x Accumax enzymes in Dubelcco's PBS (0.2g/L KCl, 0.2g/L KH<sub>2</sub>PO<sub>4</sub> ; 8g/L NaCl, 1.15g/L Na<sub>2</sub>HPO<sub>4</sub>), 0.5mM EDTA<sub>4</sub>Na, 3mg/L PhenolRed  
Accumax solution is a cell detachment solution of proteolytic, collagenasic and DNase enzymes, but does not contain mammalian or bacterial derived products.
- Storage:** Store at -20°C (shelf life of two years)  
After thawing, should be stored for up 2 months at 4°C  
DO NOT STORE AT ROOM TEMPERATURE
- Quality Control:** Each lot is controled for sterility, and for functional activity of cell detachment.
- Applications :** **Cell Lines :** Accumax has been shown effective in dissociating cell clumps in a variety of cell lines including hybridomas, CHO, BHK, 293, COS and Sf9 cells.  
**Technics :** Accumax performs exceptionally well in dissociating cell clumps for cell counting, viral transfection assays, cell sorting, and flow cytometry as well as bioreactor scale-up.

### Directions for Use

#### Cell counting

- Thaw Accumax at +37°C or room temperature
- Harvest a representative sample of clumped cells, 0.5ml or 1.0ml, and place in the container used for cell counting
- Add an equal volume of Accumax to the sample of cells, and incubate for 5 to 10 min at +37°C
- count the cells by your normal procedure. Note that the cells have been diluted an extra 2 fold
- count cells and passage as usual : no additionnal washes or enzyme inhibitor are required.

#### Primary Tissue Dissociation

This protocol for using ACCUMAX to dissociate cells from primary tissue is a general-purpose protocol and may not be applicable to all tissue types. The individual investigator needs to optimize the conditions for his/her tissue specimens. Keep in mind that ACCUMAX is a powerful enzyme mixture that can potentially dissolve not only the connective tissue of solid tissue but some fragile cell types as well if not closely monitored.

#### MATERIALS

##### *Sterile:*

- ACCUMAX
- DPBS (calcium and magnesium free)
- Culture medium, i.e., DMEM/F12 with 10 – 20% FBS (or other appropriate media)
- Pipettes-1 ml, 10 ml
- Petri dishes- 100 mm, non-tissue culture grade

For any question,  
contact you local distributor

Uptima,  
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T25 culture flasks

Centrifuge tubes, 15-50 ml, depending upon the amount of tissue being processed

Scalpels - Forceps

**Non-sterile:**

Platform rocker - Trypan Blue

Microscope – Centrifuge

**PROCEDURE:**

- Transfer the tissue to a petri dish containing fresh, sterile DPBS, and rinse.
- Transfer the tissue to a second dish; dissect off unwanted tissue, such as fat or necrotic material.
- Using two crossed scalpels or a scalpel and forceps, cut the tissue into small pieces approximately 1 mm in size.
- Transfer the tissue pieces to a 15 or 50 ml sterile centrifuge tube containing fresh, sterile DPBS.
- Allow the pieces to settle and carefully remove the supernatant. Repeat this wash step two times.
- Transfer the tissue pieces to a fresh petri dish and add enough ACCUMAX to the plate to cover tissue.
- Incubate the samples on a platform rocker at 37° C 5 to 60 minutes. The tissue will “smear” on the bottom of the dish when the disaggregation is effective. To release more cells, gently agitate the sample by pipetting several times. It is best to check cell viability several times during the incubation using Trypan blue.
- Once disaggregation is complete, transfer the cells to a sterile centrifuge tube and centrifuge at 300 x g to pellet the cells and to remove the ACCUMAX.
- Carefully remove the supernatant and re-suspend the cell pellet in 5 ml of DMEM/F12 containing 10 – 20% FBS (or other appropriate media). Seed in a T25 flask. Replace the media after 48 hours.

*ALTERNATIVELY*

- If cell isolation is from a soft tissue (such as liver) transfer the tissue to a petri dish containing fresh, sterile DPBS, and rinse.
- Transfer the tissue to a second dish; dissect off unwanted tissue, such as fat or necrotic material. Add 1 – 2 ml of ACCUMAX and use forceps to gently “tease” the cells into the ACCUMAX.
- Residual connective tissue may be separated by allowing the pieces to settle or by filtration, if desired.
- Centrifuge the sample at 300 x g to pellet the cells and to remove the ACCUMAX.
- Carefully remove the supernatant and re-suspend the cell pellet in 5 ml of DMEM/F12 containing 10 – 20% FBS (or other appropriate media). Seed in a T25 flask. Replace the media after 48 hours.

For any information, please ask :

Pour toute information complémentaire, s'adresser à :

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