



ACCUMAX™

CATALOG NUMBER: SCR006

LOT NUMBER:

QUANTITY: 100 mL

DESCRIPTION: A cell detachment solution of proteolytic, collagenolytic and DNase enzymes. Useful for creating single cell suspensions from clumped cell cultures for accurate cell counting, detachment of cells from primary tissue and the routine detachment of cells from standard tissue culture plasticware and adhesion coated plasticware, including SmartPlastic™. ACCUMAX™ does not contain mammalian or bacterial derived products.

Each lot of ACCUMAX is tested for Sterility (by USP membrane filtration method), enzymatic activity (tested with synthetic chromagenic tetrapeptides) and cell detachment from tissue culture plastic.

APPLICATIONS:

1. Cell Counting.

1. Thaw ACCUMAX at room temperature.
2. Harvest a representative sample of clumped cells, 0.5 or 1.0 ml, and place in the container used for cell counting.
3. Add an equal volume of ACCUMAX to the sample of cells, and incubate for 5 to 10 minutes at 37°C.
4. Count the cells by your normal procedure. Note that the cells have been diluted an extra 2 fold.
5. Count cells and passage as usual; no additional washes or enzyme inhibitors are required.

2. Primary Tissue Dissociation.

Recent research has revealed some unique properties of the enzymes contained in ACCUMAX. The ACCUMAX enzymes are more effective at room temperature than at 37°C. In fact, the ACCUMAX enzymes will be inactivated after two hours at 37°C. For this reason, **perform all your tissue digestion at room temperature.**

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.

Non-sterile materials required:

Platform rocker	Microscope
Trypan Blue	Centrifuge

Sterile Materials required:

ACCUMAX™	T25 culture flasks
DPBS (calcium and magnesium free)	Centrifuge tubes (15-50 ml)
Culture medium	Scalpels
Pipettes (1 ml, 10 ml)	Forceps
Petri dishes (100 mm, non-tissue culture grade)	

Procedure:

1. Transfer the tissue to a petri dish containing fresh, sterile DPBS, and rinse.
2. Transfer the tissue to a second dish; dissect off unwanted tissue, such as fat or necrotic material.
3. Using two crossed scalpels or a scalpel and forceps, cut the tissue into small pieces approximately 1 mm in size.
4. Transfer the tissue pieces to a 15 or 50 ml sterile centrifuge tube containing fresh, sterile DPBS.
5. Allow the pieces to settle and carefully remove the supernatant. Repeat this wash step two times.
6. Transfer the tissue pieces to a fresh petri dish and add enough ACCUMAX to the plate to cover tissue.
7. Incubate the samples on a platform rocker at room temperature for 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.
8. 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 cell debris if desired.
9. 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 Soft Tissue samples:

1. If cell isolation is from a soft tissue (such as liver), transfer the tissue to a petri dish containing fresh, sterile DPBS, and rinse.
2. 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.
3. Residual connective tissue may be separated by allowing the pieces to settle or by filtration, if desired.
4. Centrifuge the sample at 300 x g to pellet the cells and to remove cell debris if desired.
5. 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.



- FORMAT:** 100 ml, ready to use, frozen sterile liquid.
- PRESENTATION:** 1X ACCUMAX enzymes in Dulbecco's PBS (0.2 g/L KCl, 0.2 g/L KH₂PO₄, 8 g/L NaCl, and 1.15 g/L Na₂HPO₄) containing 0.5mM EDTA-4Na.
- STORAGE/HANDLING:** Stable when stored at -20°C. Use within one year from date of receipt. Recommended storage upon receipt is -20°C. After thawing, ACCUMAX may be stored for up to 2 months at 4°C. **DO NOT STORE AT ROOM TEMP. Use sterile technique when handling.**

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SmartPlastic™ is a registered trademark of Protein Polymer Technologies, Inc. San Diego, CA

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