



Accumax

Cell Dissociation Solution

Accumax is a ready to use non-mammalian, non-bacterial replacement for all applications of trypsin and collagenase in tissue dissociation, cell counting, and dissolving cell clumps such as spheroids.

Accumax contains the same enzymes as Accutase and is a direct replacement for collagenase.

Accumax can be used to increase the accuracy of a cell count. It can also be added to a clumpy sample on a cell sorter to extend the sort time of the sample.

Advantages of Accumax

- Dissociates tissue.
- Increases accuracy of manual or automated cell counts.
- Used to dissolve neuronal and prostate spheroids.
- Removes cells from 3-D matrixes.
- Removes cells from hollow fiber cell reactors.
- Extends the sort time of clumpy cell on a sorting flow cytometer.

Cell Lines tested

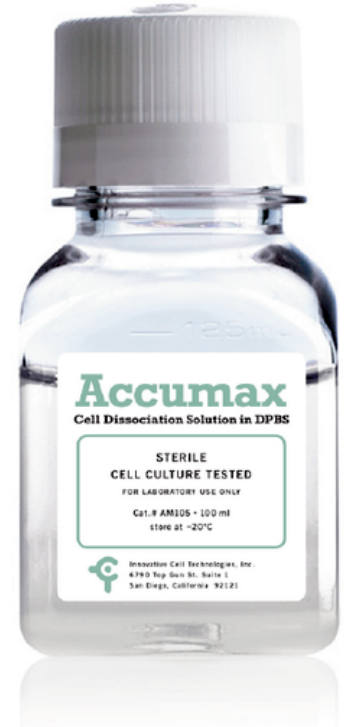
A few cell lines that **Accumax** has been shown to work on without harm:

hESCs, fibroblasts, keratinocytes, vascular endothelial cells, hepatocytes, vascular smooth muscle cells, hepatocyte progenitors, primary chick embryo neuronal cells, bone marrow stem cells, adherent CHO and BHK cells, macrophages, 293 cells, L929 cells, immortalized mouse testicular germ cells, 3T3, Vero, COS, HeLa, NT2, MG63, M24 and A375 metastatic melanoma, gliomas U251 and D54, HT1080 fibrosarcoma cells, and Sf9 insect cells.

Applications

Accumax performs exceptionally well for:

hESC culturings, analysis of cell surface markers, virus growth assay, quiescence assays by serum starvation, transformation assays by oncogenetransfection, neural crest cell migration assays, cell proliferation, apoptosis, cell haptotaxis, tumor cell migration assays, routine cell passage, production scale-up (bioreactor), and flow cytometry. Accumax is used to digest primary tissue, create single cell suspensions for cell counts, and declump cells for magnetic or flow cytometry cell sorting and removing from artificial growing matrixes.



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Accutase is a registered trademark of Innovative Cell Technologies, Inc.

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Defrosting Accutase® and Accumax Correctly

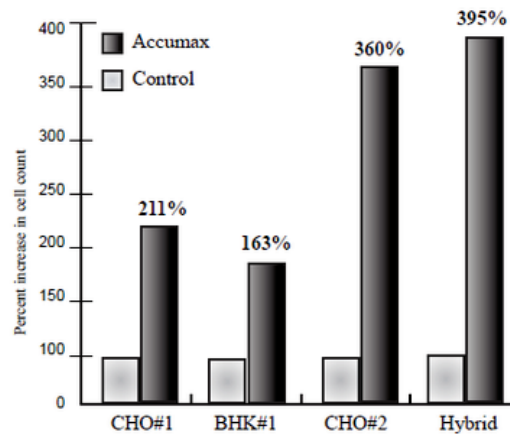
The Accutase and Accumax products are robust enzyme mixtures, when treated properly. They do not like heat. A fully frozen bottle of Accutase will be yellow in color and change to an orange color as it defrosts. This is normal. When you receive a bottle of Accutase or Accumax into your laboratory, as long as there is an ice cube floating in the solution, it has arrived in perfect condition. Once unpacked, the product can be refrozen or placed directly into the refrigerator for later use.

1. Accutase and Accumax should not be defrosted in a warm water bath. Both are sensitive to temperatures above 37°C and will be inactivated after 45 minutes at 37°C.
2. Accutase and Accumax should be defrosted overnight in the refrigerator or placed in a tub of cold tap water. Not in a 37°C water bath! It will take approximately 1-1.5 hours to defrost a bottle placed in a tub of cold water.
3. Always remember to shake up the bottle of Accutase or Accumax after defrosting. As the solution defrosts, the components will not melt evenly.
4. Although Accutase and Accumax can be defrosted, aliquoted, and then refrozen, it is not necessary. Both are stable in the refrigerator for at least 1 month. However, they should be removed from the refrigerator, used promptly, and returned to the refrigerator after use. These products do not need to be warmed up before being used.

Increasing Reproducibility of Cell Counting with the Use of Accutase

Regardless of what the manufacturers of cell counters tell you, when counting cells with either a manual or automated method, the accuracy and the reproducibility of the counts will be increased if the cells are not clumped together. Accutase solution can be used to dissociate clumpy cells that are being counted. Accutase 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.

1. Harvest a representative sample of clumped cells to be counted, 0.5 or 1.0 ml, and place in a 12x75 mm tube.
2. Add an equal volume of ACCUTASE to the sample of cells, and incubate for 5 to 10 minutes at room temperature.
3. Count the cells by your normal procedure. Note that the cells have been diluted an extra 2 fold.





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Protocol for the use of ACCUMAX™ in 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™ (Should be defrosted overnight in the refrigerator or in a bucket of room temperature water - *not a 37°C bath*)
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
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:

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** 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.



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Primary Tissue Dissociation Protocol, cont. page 2

ALTERNATIVELY

If cell isolation is from a soft tissue (such as liver):

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. 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.