Protocol for the use of ACCUMAX™ in Primary Tissue Dissociation

Recent research at ICT has revealed some unique properties of the enzymes contained in Accumax. The Accumax enzymes actually are more effective at room temperature than at 37C. In fact, the Accumax enzymes will be inactivated after two hours at 37C. For this reason, do 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.

MATERIALS

Sterile:
ACCUMAX™ (Should be defrosted overnight in the refer or in a bucket of room temperature water-not a 37C 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:

- 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 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.

- 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.

- 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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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 cell debris if desired.

• 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.