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)

- Accmax (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 D-PBS, 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 D-PBS.
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 - 60 minutes. The tissue will "smear" on the bottom of the dish when the disaggregation is effective.
   o To release more cells, gently agitate the sample by pipetting several times.
   o 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 resuspend 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):

1. Transfer the tissue to a petri dish containing fresh, sterile D-PBS, 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 resuspend 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.