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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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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.