Tissue Dissociation SOP

Flow cytometry facilitates the analysis of millions of cells within a short period of time. Primary cells are generally harvested from tissues and must be dissociated and filtered to yield a single cell suspension prior to staining and Flow cytometric analysis. Ideally, the dissociation method must consistently yield viable cells without altering phenotype or function. There are 3 main methods for liberating cells from tissue and all have benefits and limitations. These include: chemical, mechanical and enzymatic dissociation.

Chemical dissociation: cations are required for cell to cell ligand mediated binding. Chemicals such as EDTA or EGTA (0.1-1.0 mM) are chelators for divalent cations, disrupting cell to cell adhesion. EDTA is a pan chelator of divalent cations: Mg++, Mn++, Zn++, and Ca++, whilst EGTA preferentially binds Ca2+.

*NB these chelators inhibit enzymes that require Ca++ for activity such as Collagenase and Dispase

Mechanical dissociation: involves cutting, scraping or scratching the tissue in order to fragment into small pieces. Gentle agitation such as trituration may then be applied to loosen the cells. The fragments are then washed and filtered. These conditions are sufficient to dissociate cells of the thymus and spleen.

Enzymatic dissociation: enzymes are used to digest tissues in order to liberate cells into a single cell suspension. Many different types of enzymes are available that can be used alone or in combination. Enzymes have various specificities that make them more effective with certain tissues, so it is best to determine which enzymes are best suited for the tissue of interest. Enzyme concentrations, temperature and incubation times should always be considered for maximum viability.

Table 1. Digestive enzymes in solid tissue disaggregation

ENZYME	PURPOSE
Dispase	-Breaks down extracellular matrix -Detaches cell colonies -Cleaves attachments between cells and extracellular matrix
Collagenase	-Breaks down extracellular matrix -Breaks peptide bonds present in collagen
Hyaluronidase	-Breaks down extracellular matrix -Cleaves glycosidic bonds in hyaluronan
Papain	-Degrades proteins which make up tight junctions between cells
DNase-I	-Degrades free-DNA -Prevents cell aggregation
Accutase	-Proteolytic, collagenolytic, and DNase activity
TrypLE	-Cleaves cell–cell junctions -Does not alter antigen expression as trypsin would

Reference:

Cytometry Part A 95A: 219–226, 2019, Best Practices for Preparing a Single Cell Suspension from Solid Tissues for Flow Cytometry, Reichard A and Asosingh K

https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.23690

Benefits
Specific enzymes work well on certain tissues.

Limitations
Enzyme dissociation can modify/cleave proteins on the cell surface, which can affect cell function or binding of fluorescent antibodies. Enzyme dissociation can also be more time consuming compared with mechanical dissociation.

Commonly used enzymes and some general protocols:

Trypsin: serine protease used for cell detachment and tissue dissociation.

General protocol:

1. Mince tissue into 3 to 4 mm pieces with a sterile scalpel or scissors. Wash the tissue pieces several times with PBS.

2. Add trypsin and iIncubate at 37°C for 5-10 min.
3. Neutralise trypsin using PBS containing 2% serum.
4. Wash suspension by centrifugation in PBS.
5. Resuspend the pellet in appropriate medium and filter.

Collagenase: hydrolyses collagen, widely used to isolate cells from animal tissue. Different isoforms exist with varying specificities.

General protocol:

1. Mince tissue into 3 to 4 mm pieces with a sterile scalpel or scissors. Wash the tissue pieces several times with Hanks' Balanced Salt Solution (HBSS).
2. Add collagenase (50 to 200 U/ml in HBSS) and incubate at 37°C for 4 to 18 h. Addition of 3 mM CaCl2 increases the efficiency of dissociation. Filter the cell suspension. Fresh collagenase can be added to the fragments if further disaggregation is required.
3. Wash suspension by centrifugation in HBSS.
4. Resuspend the pellet in appropriate medium and filter

https://www.thermofisher.com/au/en/home/references/protocols/cell-culture/primary-cell-protocols/dissociation-of-cells-from-primary-tissue.html

Dispase II: hydrolyzes collagen bonds. It is a gentle enzyme, dissociates well at physiological temperature and pH, and maintains cell membrane integrity.

General protocol:

1. Mince tissue into 3 to 4 mm pieces with a sterile scalpel or scissors. Wash the tissue pieces several times in a calcium and magnesium-free balanced salt solution.

2. Add dispase (0.6 to 2.4 U/ml in calcium and magnesium-free balanced salt solution) and incubate at 37°C for 20 min to several hours.

3. Filter the cell suspension. Fresh dispase can be added to the fragments if further disaggregation is required.

4. Wash suspension several times by centrifugation in the balanced salt solution.

https://www.thermofisher.com/au/en/home/references/protocols/cell-culture/primary-cell-protocols/dissociation-of-cells-from-primary-tissue.html

Accutase: an enzymatic mixture with proteolytic and collagenolytic enzyme activity. It mimics the action of trypsin and collagenase at the same time.

https://www.amrepflow.org.au/public/documents/StemProA11105-01Accutasegibcolifetech.pdf

A concentrated form of Accutase:

Accumax (Should be defrosted overnight in the refrigerator or at room temperature water - not at 37 °C)

General Protocol:

1. Rinse tissue in fresh, sterile DPBS.
2. Mince tissue into 1 to 4 mm pieces with a sterile scalpel or scissors.
3. Transfer the tissue pieces to a 15 or 50 ml sterile centrifuge tube containing fresh, sterile DPBS.
4. Allow the pieces to settle and carefully remove the supernatant. Repeat this wash step two times.
5. Transfer the tissue pieces to a fresh tube and add enough Accumax to cover tissue.
6. 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 by trituration several times. It is best to periodically check cell viability during the incubation using Trypan blue.
7. Once disaggregation is complete, wash cells in PBS.

8. Carefully remove the supernatant and re-suspend the cell pellet in appropriate medium and filter.

Alternative protocol:

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

1. Wash tissue in fresh, sterile DPBS.
2. Transfer the tissue to a petri 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. Wash cells in PBS
4. Re-suspend the cell pellet in appropriate medium and filter.

Reference: Innovative cell technologies

https://www.amrepflow.org.au/public/documents/primary_cell_dissocaition_protocol_for_accumax.pdf

https://www.amrepflow.org.au/public/documents/SCR006accumax.pdf

Papain: Papain has a wide specificity. It degrades protein substrates such as cartilage and typically used to digest neural tissue

1. Mince tissue into 3 to 4 mm pieces with a sterile scalpel or scissors. Wash the tissue pieces
2. Incubate with activated papain at 37°C, followed by trituration.
3. Dissociated cells are pelleted then resuspended in medium containing ovomucoid, a papain inhibitor.
4. Wash cells in PBS
5. Re-suspend the cell pellet in appropriate medium and filter.

http://www.worthington-biochem.com/PDS/default.html

Enzymes that can be used in combination with collagenase include:

Hyaluronidase: For hydrolysis of hyaluronic acid

Elastase: Useful for the dissociation of tissues containing higher amounts of elastin, a substrate not hydrolysed by trypsin or pepsin. Elastin is found in the elastic fibers of connective tissues.

Protease Type XIV (Pronase E): Nonspecific protease for dissociation of various tissues.

There are also many tissue dissociation kits available:

https://www.miltenyibiotec.com/US-en/products/macs-sample-preparation/tissue-dissociation-kits.html?gclid=CjwKCAjw7-P1BRA2EiwAXoPWA4WGEIrh90437VVH0_DZhibA_WfN3BTa7iQF_SIcU5qLxUd2vvxXqBoCm8MQAvD_BwE

https://cdn.stemcell.com/media/files/techbulletin/TB29107-Guide_to_Solid_Tissue_Dissociation.pdf