Preparing a Single Cell Suspension from Either Frozen or Primary Tissue Samples

Preparation of a single cell suspension is critical for successful cell isolation. Performing cell separation on samples containing cell clumps will result in cell loss during isolation and may interfere with proper labeling of the target cells.

The following protocols outline how to harvest cells and prepare single cell suspensions from either frozen or primary tissue samples prior to performing cell separation:

Protocol for Frozen Samples

1. Thaw the vial of cells quickly by swirling it in a 37 ° C water bath. Transfer thawed cells into a sterile 50 mL conical tube.
2. Rinse the vial with 1 mL of culture medium containing 10 % FBS to recover any remaining cells, and transfer the medium to the new tube.
3. Top up the 50 mL tube with culture medium containing 10 % FBS. Gently invert to mix.
4. Centrifuge the 50 mL tube at 300 x g for 10 minutes at room temperature to collect the cells. (G to RPM Converter)
5. Carefully remove and discard as much of the supernatant as possible, taking care to not disturb the pelleted cells. Gently tap the tube to resuspend the pellet.
6. If cells appear clumpy, calculate the volume of DNase I that should be added to the sample to yield a final concentration of 100 µg/mL. Add DNase I drop-wise to the cell suspension while gently swirling the tube. Incubate for 15 minutes at room temperature.
7. Add 25 mL of culture medium containing 2 % FBS, gently invert to mix and centrifuge as above. Discard as much of the supernatant as possible and gently resuspend the pellet.
8. If cells still appear clumpy, pass the sample through a 30 - 70 µm mesh cell strainer into a fresh conical tube. Rinse the sample tube three times with culture medium containing 2 % FBS and pass through the strainer.
9. The cell suspension is now ready for cell counting and further downstream applications such as cell isolation.

Protocol for Primary Tissue Samples

When working with primary tissue samples, cells must be dissociated from the primary tissue sample in order to prepare a single cell suspension .

1. In a sterile dish containing culture medium and/or dissociation enzyme, mince the harvested tissue. Please contact us or review the current literature for appropriate reagent selection and more detailed procedures for digesting specific tissue types.
2. Pass the sample through a 70 µm or 100 µm mesh cell strainer into a fresh conical tube to further dissociate

the cells.

3. Gently pipette 2-3 mL of culture medium over the tissue and cells remaining in the strainer. Repeat this step and then discard the leftover tissue and strainer.
4. Add culture medium - up to 40 - 50 mL total volume - to the tube and collect cells by centrifugation. Please contact us or review current literature protocols to determine an appropriate culture medium and centrifugation speed for your sample.
5. Carefully remove and discard as much of the supernatant as possible, taking care to not disturb the pellet. Gently resuspend the pellet.
6. (Optional wash step) Carefully add 25 mL of culture medium to the cell pellet, gently invert to mix and centrifuge as above. Discard as much of the supernatant as possible and gently resuspend the pellet.
7. The cell suspension is now ready for cell counting and further downstream applications such as cell isolation.