Ethanol Fixation Protocol

Reagents:

- 70% Ethanol (e.g. Sigma E7023, in PBS or distilled water) - Store at -20°C
- Ice cold Ca2+-free and Mg2+-free PBS (e.g. Phosphate Buffered Saline (PBS, e.g. Life Tech #10010-23)

Protocol:

Fixation:

1. Pellet cells (300xg, 5min) and remove supernatant (Note: if you are detaching adherent cells, pipette triturate in detachment media (e.g. Accutase) to break up clusters. (Note: Put pipettor on a lower setting to avoid excess trauma to cells)
2. Wash the cells twice in PBS (300xg, 5min, 4°C). Count the cells when finished.
    a. *Note 1:* Recommend a total of 5e5-2.5e6 cells so that the re-suspension is 1-5e6/ml (when adding 0.5ml final re-suspension later).
    b. *Note 2:* Put PBS on ice during centrifugations to keep cold.
    c. *Note 3:* Pipette triturate 10-20x when re-suspending pellet to ensure single cell suspension. Preferred approach is add ~1mL PBS to pellet and use a 1mL pipette to triturate. Then add remaining 4ml PBS for wash. Invert 3x.
3. Re-suspend the pellet in approximately 500 ul of ice-cold PBS. Pipet with 1000µL pipette, up and down, 20 times. It is important that this be a good single-cell suspension at this point, or the cells will be fixed as clumps.
4. Aliquot 4.5mL of ice cold 70% ethanol to a 15mL centrifuge tube.
5. Hold ethanol tube and cell tube in a cold pack and vortex gently.
6. Add .5ml cells drop-wise to the 70% ethanol tube (while vortexing) using a 100uL pipette (max size to ensure drop volume is small).
7. Place in Freezer for 1hr to 4days

Post-Fixation Recovery/Use:

1. Centrifuge fixed cells at 800xg, 10°C, 5min with BRAKE OFF. Remove the ethanol.
2. Re-suspend in 1ml ice-cold PBS with 20x pipette trituration (1000uL pipette)
3. Add 4mL ice-cold PBS and invert 3x.
4. Centrifuge at 700xg, 10°C, 5min with BRAKE OFF. Remove the supernatant.
5. Re-suspend in 1ml ice-cold PBS with 20x pipette trituration (1000uL pipette)
6. Add 4mL ice-cold PBS and invert 3x
7. Centrifuge at 600xg, 10°C, 5min with BRAKE OFF. Remove the supernatant.
8. Re-suspend the pelleted cells in in desired solution for staining