Procedure for isolation for cells from Fat (mesenchymal stem cells and immune cells)**:**

1. Weigh tissue (fat) on a Petri dish.
2. Using the scissors mince tissue with scissors as fine as possible (yogurt consistency). Mince a max of 3 ml of tissue
3. Using a spatula pour minced tissue to a new 50 ml conical tube. Pour tissue directly into tube from Petri dish.
4. Pipette 10ml of 0.075% Collagenase Type 1.* Use collagenase solution to rinse the Petri dish that was used to mince fat before adding to 50 ml tube.
5. Incubate for 1 hr at 37˚C on mixer in an incubator.
6. Inactivate collagenase by adding an equivalent volume of complete media (usually 10ml)
7. Vortex.
8. Centrifuge the 50 ml conical tube for 10 min at 1200 rpm. Cells of interest are in pelleted fraction.
9. Using a vacuum aspirator, discard media from the 50 ml conical tube leaving as little media as possible.
10. Resuspend pellet in 10 ml erythrocyte lysis buffer (154mM NH_4Cl, 10mM $KHCO_3$, 1mM EDTA). Pipette up and down a few times to break up the pellet.
11. Run resuspended cells through 100µm strainer, placed over the new 50ml tube.
12. Incubate at room temperature for 10 minutes.
13. Centrifuge for 10min at 1200 rpm. Discard supernatant.
14. Resuspend the "invisible" pellet in 500 ul WASH buffer (0.04% BSA in PBS).
15. Filter cells through 40 µm FLOWMI Cell Strainer (removes adipose cells).
16. Measure viability/cell number.
17. In case there is debris spin down a couple times (300xg for 5 minutes) and dissolve in appropriate volume WASH buffer (700-1,200 cells/µl)

*The amount of collagenase solution added depends on the amount of tissue received. If the amount of tissue is greater than 10g add additional 5-10 ml of 0.075% Collagenase Type 1.