## Human Adipose Dissociation and Cell Culture

**Purpose:** To dissociate human adipose to obtain adipose progenitors and endothelial cells for cell culture experiments.

**I. Materials:**

**Adipose Tissue Dissociation Kit mouse and rat (Miltenyi Biotech; 130-105-808)**
Kit components: 5 vials, containing:
2 vials of Enzyme D (lyophilized powder)
1 vial of Enzyme R (lyophilized powder)
1 vial of Enzyme A (lyophilized powder)
1 mL of Buffer A

**Fibroblast Media (made ahead of time)**
500mL MEMα (Gibco;32561037)
10 µL EGF 500 µg/mL (R&D Systems; 263-EG-200 = recon in 400 µL PBS, aliquot and store at -80C )
2.5 µL FGFbasic 50 µg/mL (R&D Systems; 233-FB-025 = recon in 500 µL PBS, aliquot and store at -80C)
50 mL FBS
5 mL MEM Non-essential amino acids (NEAA) (Gibco;11140050)
5 mL Pen/Strep (Gibco;15140122)

**II. Reagent preparation**
1. Prepare **Enzyme D** by reconstitution of the lyophilized powder in each vial with 3 mL of DMEM and sterile filter. Prepare 0.5 mL aliquots to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution.
2. Prepare **Enzyme R** by reconstitution of the lyophilized powder in the vial with 2.7 mL of DMEM. Prepare 250 µL aliquots to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution.
3. Prepare **Enzyme A** by reconstitution of the lyophilized powder in the vial with 1 mL of **Buffer A** supplied with the kit. Do not vortex. Prepare 50 µL aliquots to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution.

**III. Adipose tissue dissociation protocol**
1. Prepare **Enzyme Mix** with prepared enzymes from step 1. 2-4 samples are typically processed at a time and enzyme mix should be prepared as master mix for all the samples.
**Enzyme Mix**:

| **Component** | **(1X)** | **X** |
|---|---|---|
| DMEM | 2.35 mL | |
| Enzyme D | 100 µL | |
| Enzyme R | 50 µL | |
| Enzyme A | 12.5 µL | |
| **Total** | **2.5 mL** | |

2. Aliquot 2.5 mL of **Enzyme mix** into gentleMACS C Tube for each sample and label tube.
3. Transfer 0.5g of adipose tissue into the gentleMACS C Tube containing the enzyme mix. Use a small sterile scissors to cut tissue into small pieces while in the C Tube. Tightly close tube beyond the first resistance.
4. Use the heating function of the **gentleMACS Octo Dissociator with Heaters** run program **37C_mr_ATDK_1.**
**5.** After termination of the program, detach C Tube from the gentleMACS Dissociator.
6. **(Optional)** Perform a short centrifugation step up to 300×g to collect the sample material at the tube bottom and resuspend cells.
7. Label a 15 mL tube for each sample. Place a MACS SmartStrainer (100 µm) on each 15 mL tube.
8. Pour the cell suspension through the MACS SmartStrainer (100 µm) into the labeled 15 mL tube.

9. Rinse the C tube and wash the MACS SmartStrainer with 5–10 mL of DMEM for each sample.
10. Discard the MACS SmartStrainer and centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
11. Gently resuspend cell pellet with 1 mL ACK Lysis Solution (Gibco A10492-01) to lyse red blood cells. Incubate samples at room temperature for 3 minutes and add 3 mL DMEM to each sample.
12. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant leaving ~0.5mL.
13. Resuspend cell pellet and transfer to labeled 10cm plate with 10 mL **Fibroblast media** for each sample.

**IV. Cell culture**

1. Place 10 cm plates in 37C/5% CO2 incubator.
2. Change media on the cells after 18-24 hours and change the media every 3-4 days until confluent using Fibroblast media.
3. Harvest cells by removing media and adding 2 mL TrypLE to 10 cm plate. Incubate at room temperature until cells begin to detach.
4. Resuspend cells and transfer to 15 mL tube with 2 mL Fibroblast media.
5. Centrifuge cells at 1500 rpm for 3 minutes. Remove supernate and resuspend cells in residual volume.
6. Transfer 1/10 of cells to new 10 cm plate with 10 mL Fibroblast media to continue to growing.
7. Freeze down the remainder of the cells in fibroblast freezing media (50% FBS/40% Fibroblast media/10% DMSO) in 1 mL aliquots (2-3 aliquots/10cm plate)

Protocol adapted from the Miltenyi Biotech Adipose Tissue Dissociation Kit mouse and rat kit protocol. ELT