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Human Adipose Dissociation and Cell Culture -- University of Minnesota TMCs

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Cellular Senescence Network (SenNet) Method Development Community

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ABSTRACT

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

Human Adipose Dissociation and Cell Culture (2).pdf

Adapted from Miltenyi Biotec - Adipose Tissue Dissociation Kit (attached).

Miltenyi Adipose dissociation protocol.pdf

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Protocol status: Working
We use this protocol and it's working

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I. Materials

1 **Adipose Tissue Dissociation Kit mouse and rat (Miltenyi Biotec; 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

1.1 **Fibroblast Media (made ahead of time)**
500mL MEMα (Gibco; 32561037)
10µL EGF 500 µL/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

2 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.

3 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.

4 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

5 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	

6 Aliquot 2.5 mL of Enzyme mix into gentleMACS C Tube for each sample and label tube.

7 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.

8 Use the heating function of the **gentleMACS Octo Dissociator with Heaters** run program **37C_mr_ATDK_1**.

9 After termination of the program, detach C Tube from the gentleMACS Dissociator.

10 **(Optional)** Perform a short centrifugation step up to 300×g to collect the sample material at the tube bottom and resuspend cells.

11 Label a 15 mL tube for each sample. Place a MACS SmartStrainer (100 µm) on each 15 mL tube.

12 Pour the cell suspension through the MACS SmartStrainer (100 µm) into the labeled 15 mL tube.

13 Rinse the C tube and wash the MACS SmartStrainer with 5–10 mL of DMEM for each sample.

14 Discard the MACS SmartStrainer and centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.

15 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.

16 Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant leaving ~0.5mL.

17 Resuspend cell pellet and transfer to labeled 10cm plate with 10 mL **Fibroblast media** for each sample.

IV. Cell Culture

18 Place 10 cm plates in 37C/5% CO2 incubator.

19 Change media on the cells after 18-24 hours and change the media every 3-4 days until confluent using Fibroblast media.

20 Harvest cells by removing media and adding 2 mL TrypLE to 10 cm plate. Incubate at room temperature until cells begin to detach.

21 Resuspend cells and transfer to 15 mL tube with 2 mL Fibroblast media.

22 Centrifuge cells at 1500 rpm for 3 minutes. Remove supernate and resuspend cells in residual volume.

23 Transfer 1/10 of cells to new 10 cm plate with 10 mL Fibroblast media to continue to growing.

24 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)