Oct 06, 2023

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

DOI
https://dx.doi.org/10.17504/protocols.io.eq2lyj8jmlx9/v1

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Cellular Senescence Net...

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Protocol Citation: Elizabeth Thompson, Laura J Niedernhofer, David A Bernlohr 2023. Human Adipose Dissociation and Cell Culture -- University of Minnesota TMCs. **protocols.io** https://dx.doi.org/10.17504/protocols.io.eq2lyj8jmlx9/v1

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

Created: October 05, 2023

Last Modified: October 06, 2023

Protocol Integer ID: 88871

Keywords: human adipose, adipose tissue, dissociation, adipose tissue dissociation kit, human adipose dissociation, adipose progenitor, endothelial cells for cell culture experiment, cell culture, cell culture experiment, endothelial cell, cell, miltenyi biotec

Funders Acknowledgements:
NIH
Grant ID: 5U54AG079754-02
NIH
Grant ID: 5U54AG076041-03

Abstract

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

Human Adipose Dissociation and C... 50.5KB

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

Miltenyi Adipose dissociation proto... 77.2KB

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	

	Component	(1X)	X
	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)