Immune cell isolation from adipose tissue

(this protocol is modified on the Adipose Tissue Dissociation Kit instructions)

Reagent preparation

Prepare Enzyme D by reconstitution of the lyophilized powder in each vial with 3 mL of DMEM. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C.

Prepare Enzyme R by reconstitution of the lyophilized powder in the vial with 2.7 mL of DMEM. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. (Note: Make sure to thoroughly mix this suspension immediately before withdrawing the required reaction volume)

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 aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C.

Adipose tissue dissociation protocol

Appropriate volume of enzyme mix based on tissue volume:

	White adipose tissue	**Brown adipose tissue**
Up to 0.5 g tissue	**2.5 mL**	**1.25 mL**
0.51–1.0 g tissue	**5 mL**	**2.5 mL**

For mPVAT (0.4 g-0.5 g), prepare enzyme mix by adding 2.35 mL of DMEM, 100 µL of Enzyme D, 50 µL of Enzyme R, and 12.5 µL of Enzyme A into a gentleMACS C Tube for a dissociation volume of 2.5 mL.

Prepare clean tweezers and scissors (sterilize with ethanol).

Resect the adipose tissue and cut it into small pieces of 2–4 mm.

Transfer the tissue into the gentleMACS C Tube containing the enzyme mix and tightly close it.

Incubate sample for 20 minutes at 37 °C under continuous agitation using the Rotisserie rotator.

Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.

Run the gentleMACS Program mr_adipose_01.

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

Incubate sample for 20 minutes at 37 °C under continuous rotation using the Rotisserie rotator.

Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.

Run the gentleMACS Program mr_adipose_01.

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

Perform a short centrifugation step up to 300×g to collect the sample material at the tube bottom.

Resuspend sample and apply the cell suspension to a cell strainer (100 µm) placed on a 50 mL tube.

Wash cell strainer (100 µm) with 5–10 mL of DMEM.

Discard the cell strainer (100 µm) and centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.

Resuspend cells with an appropriate buffer to the required volume for further applications, for example, resuspend cells in PBS buffer for flow cytometry.