Preparation of conditioned medium from Rat Adipose Tissue

(this protocol is modified on the Jamie's protocol)

Label 5ml culture tube for each sample and pre-chill on ice

Prepare clean tweezers (sterilize with ethanol)

Add 500ul DMEM into the tube

Transfer the fat sample into the 5ml culture tube

While keeping tube on ice, carefully insert TissueRuptor tip into tube at slow speed starting at surface and working down for 15 sec. Increase to half speed for additional 20 sec.

Pull tip of TissueRuptor out of liquid and allow to spin at half speed for 10 sec to "rinse" the tip of homogenizer

Leave the tube on ice for 10 min.

——————————————————Following steps in the hood——————————————————

Label 6-well culture plate

Place 500ul of DMEM in pool at the center of the well

Place a Millipore Millicell filter insert in well and let it contact the DMEM at the bottom

Add the homogenate into the insert, close the lid of the 6-well plate and wrap outside edge of plate with parafilm

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Place plate in 37C incubator for 10 min

Foil-wrap the plate and place plate on rotator shaker between 150-200 rpm for 1 hour

Check after 30 min, change insert in the hood if it is clogged

After 1 hour, check the homogenate in the insert to see if most has gone through the filter (may need more time)

Collect liquid in the well and the residual fluid at the bottom of the insert into a 15 ml conical tube

Centrifuge the tube at 2500xg for 1 min at RT

Transfer the supernatant in a new 15 ml conical tube

Centrifuge the tube at 2500xg for 5 min at RT

Collect the final supernatant into 2 mL tube and run a BCA protein assay to determine the concentration

Store the conditioned medium in 2mL tube in -80c freezer