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MDM culture

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ASAP Collaborative Rese… West lab protocols

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We use this protocol and it's working

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Abstract

This protocol details the steps for isolation of PBMC from donors' buffy coat and differentiation protocol into MDM cells. We use this MDM model to investigate the immune cellular uptake and proinflammatory response upon treatment with alpha-synuclein fibrils.

Materials

Peripheral blood mononuclear cells (PBMCs) were acquired through separation from the buffy coat collection from the generous donation from the Red blood cross center.

Protocol materials

Lymphoprep™ **STEMCELL Technologies Inc. Catalog #**07801 Step 3

SepMate™-50 (IVD) 100 Tubes **STEMCELL Technologies Inc. Catalog #**85450 Step 3

polystyrene round-bottom tube **STEMCELL Technologies Inc. Catalog #**38007 Step 8

EasySep™ Human Monocyte Enrichment Kit without CD16 Depletion For processing 1 x 10^9 cells **STEMCELL Technologies Inc. Catalog #**19058 Step 9

1X Dulbecco's Phosphate Buffered Saline (DPBS) **Thermo Fisher Scientific Catalog #**14190094 Step 2

FBS **Atlanta Biologicals Catalog #**S11150H Step 2

1 Use the buffy coat transported from Red blood cross center (On ice , Overnight).

2 Dilute blood with an equal amount of
1X Dulbecco's Phosphate Buffered Saline (DPBS) **Thermo Fisher Scientific Catalog #**14190094
with 2% Fetal Bovine Serum (PBS + 2% FBS **Atlanta Biologicals Catalog #**S11150H)

3 Transfer the blood on top of Lymphoprep™
Lymphoprep™ **STEMCELL Technologies Inc. Catalog #**07801 in the 50-ml
SepMate™-50 (IVD) 100 Tubes **STEMCELL Technologies Inc. Catalog #**85450

4 Centrifuge at 1200 x g for 00:20:00 Room temperature (15 - 25°C), with the brake on. If the blood has been stored for more than 2 hours, increase the centrifugation time to 00:30:00 . 50m

5 Pour off the top layer, which contains the enriched MNCs, into a new tube. Do not hold the SepMate™ tube in the inverted position for longer than 2 seconds.

6 Wash enriched PBMCs with PBS + 2% FBS. Repeat wash with 400 x g for 10 minutes at room temperature.

7 Prepare the PBMCs at the 5 x 10^7 cells/ml within the 1 mL of PBS + 2% FBS buffer.

8 Add the sample to the 5 mL
polystyrene round-bottom tube **STEMCELL Technologies Inc. Catalog #**38007

9 Add enrichment Cocktail to sample at 50 ul/ml from the EasySep™ Human Monocyte Enrichment Kit without CD16 Depletion
EasySep™ Human Monocyte Enrichment Kit without CD16 Depletion For processing 1 x 10^9 cells **STEMCELL Technologies Inc. Catalog #**19058
(Cat: 19058C.2). Pipet up and down for at least 5 times. Make sure you see no clump in bare eyes. It must be mixed very thoroughly, or your yield will below.

10 Incubate On ice for 00:10:00 10m

11 Add 50 µL mag beads buffer (from STEMCELL TECH KIT, make sure you VORTEX the mag beads buffer 30 secs before taking 50 µL out!!!) and pipet up and down for no more than 5 times (enough to see the beads mixed all well (determined by the color).

12 Incubate the mix in the refrigerator or on the ice for 00:05:00 5m

13 Transfer the mix into either sterile flow tube or 15 ml canonical tube (depend on which mag selection set you would use. If you want to use the single mag set, then you have to use sterile flow tube. The multiple
channel mag set would allow you to choose between flow tube or 15 ml canonical tube.

14 Add the selection buffer up to 2.5 ml (For flow tubes, add 2.5 mL PBS or up to 2.5 mL marker on the tube).

15 Hold the tube (if you use the multiple channel mag set) and pour the mix buffer out into the new collection tube (15 ml canonical tube) in a continuous manner (don't let the mix buffer back flow to contact the mag
beads trapped by the mag set on the tube wall). Don't try to drip out the last drop of the buffer. You would rather wash twice instead of shaking of the beads to contaminate your final yield.

16 Add another 2.5 ml of selection buffer and repeat the steps listed above. Take 10 ul to mix with 90 ul of typan blue in PBS for cell counting (formula here: Total cells you count in four chamber/4x10x5x10^4 =total cells number in 5 ml buffer).

17 Centrifuge at 400 x g , 00:15:00 , Room temperature , with full break and remove the selection buffer supernatant thoroughly (to remove EDTA) and resuspend with DMEM+10%FBS+1XGlutmax+ 1X Antianti+20 ng/ml human recombinant MCSF (1000x; stock should stored at-80 C). All the cytokines are from PeproTech and should be dissolved in stock 1000x except CCL2 and IL34 (100x). 15m

18 Seed the cells into the cell culture plates (2.5 X 10^5/well for 24-well plate with 1 ml of cell culture medium

19 Incubate the cell culture in CO2 cell incubator. At day 3 add 0.5 mL additional medium containing MCSF to cell culture.
MDM should be ready to use at day 7 (the cell maturation different from patient to patient, 7 days will be a safe place to use. For most of patients, if the MDM cells still don't grow well up to

10 days, it won’t.)

Don’t let the MDM grow more than 12 days (or the media will become yellow)