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MDM culture

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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.

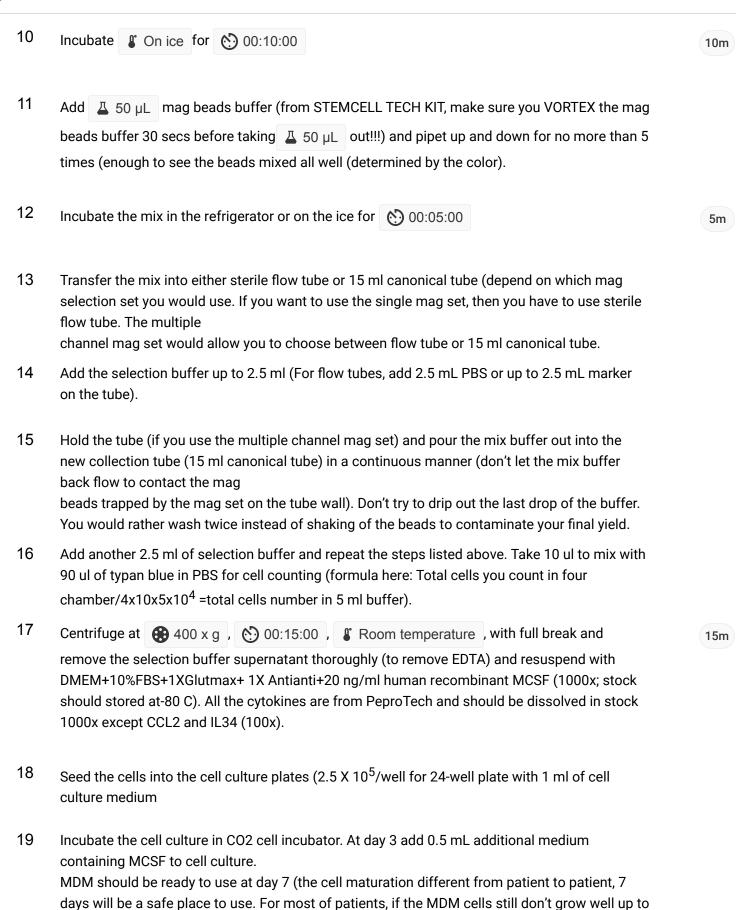
Protocol materials



FBS Atlanta Biologicals Catalog #S11150H



1 Use the buffy coat transported from Red blood cross center (F On ice , 🚫 Overnight). 2 Dilute blood with an equal amount of 1X Dulbecco's Phosphate Buffered Saline (DPBS) Thermo Fisher
Scientific Catalog #44400001 Scientific Catalog #14190094 with 2% Fetal Bovine Serum (PBS + 2% | Stanta Biologicals Catalog #S11150H) 3 Transfer the blood on top of Lymphoprep™ X 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 600:20:00 Room temperature (15 - 25°C), with the brake 50m on. If the blood has been stored for more than 2 hours, increase the centrifugation time to **(*)** 00:30:00 . 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 q for 10 minutes at room temperature. 7 Prepare the PBMCs at the 5 x 10⁷ cells/ml within the 4 1 mL of PBS + 2% FBS buffer. 8 Add the sample to the 4 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 days, it won't.)

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