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Viable PBMCs isolation using density-gradient separation

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

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Keywords: high quality pbmcs from whole blood, gradient separation collection of pbmc, viable pbmcs isolation, alignment across ongoing parkinson, biomarker development, gradient separation collection, high quality pbmc, pbmc, ongoing parkinson, incorporating sepmate tube, stem cell technology, different lab, density gradient method, sepmate tube, blood collection

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Abstract

Collection of PBMCs for biomarker development is an essential part of translational research. Several methods are employed by different labs, including our own. Here we describe in detail, a method (in place in our lab from 2025) to isolate high quality PBMCs from whole blood (EDTA blood) within ~45 minutes from blood collection. We use a density gradient method, incorporating SepMate tubes (Stem Cell Technologies). We would like to share this protocol for the purpose of standardization, comparison and alignment across ongoing Parkinson's disease cohort studies.

Guidelines

Working with blood. Before starting, make sure that all local safety and occupational health rules are followed. For example, vaccinations, safety instructions.

Materials

Guidelines

Except for the centrifugation steps, all sample processing was carried out under a biosafety cabinet in sterile conditions.

Store Lymphoprep, CryoStor CS10, PBS+2%FBS and Erylysis buffer at 4°C.

Before start

Whole peripheral blood samples are collected in EDTA tubes by professional healthcare workers in medical examination rooms.

Prepare 100mL of PBS+2%FBS for two blood vials (~15mL) under the hood.

Prepare 50mL of Erylysis Buffer as follows:

- 0.1mM EDTA
- 155mM NH_4Cl
- 10mM $KHCO_3$
- Adjust the pH to 7.3-7.4 with HCl or NaOH

Bring Lymphoprep, PBS+2%FBS solution, Erylysis buffer, CryoStor CS10 and Mr. Frosty at room temperature (18°C-25°C).

Materials

SepMate™ tubes (50mL) for density gradient separation **STEMCELL Technologies Catalog #85450**.

Falcon tubes (50mL) **Corning Catalog #352070**

Falcon Pipettes (25mL) **Corning Catalog #357525**

Falcon Pipettes (10mL) **Corning Catalog #356551**

Falcon Pipettes (5mL) **Corning Catalog #357543**

Cryo. S (2mL) **Greiner Bio-One Catalog #122263**
Lymphoprep™ **STEMCELL Catalog #07851/07861**
CryoStor CS10 **STEMCELL Catalog #07930**
1X Dulbecco's Phosphate Buffer
Saline **Sigma-Aldrich Catalog #D8537**
Fetal Bovine Serum, Premium **Thermofisher Catalog #A5670701**

Protocol materials

- Lymphoprep™ 500 mL **STEMCELL Technologies Inc.** **Catalog #**7851
- Dulbecco's Phosphate Buffer Saline **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**D8537
- Fetal Bovine Serum, Premium **Gibco - Thermo Fisher Scientific** **Catalog #**A5670701
- CryoStor CS10-100ml **STEMCELL Technologies Inc.** **Catalog #**07930
- 50ml Falcon tubes **Corning** **Catalog #**352070
- SepMate-50 **STEMCELL Technologies Inc.** **Catalog #**85450
- DMSO **Panreac AppliChem** **Catalog #**A3672
- CRYO.S 2 ML PP ROUND BOTTOM INTERNALTHREAD **greiner bio-one** **Catalog #**122263-2DG

Safety warnings

Please wear protective clothing, PPE and follow local safety guidelines.

Ethics statement

All research must adhere to the current, updated Helsinki declaration.
Prior ethical approval must be granted.
Informed consent must be given.

Before start

Ethics approvals, consent and local safety rules in place.
See section preparation below.

Preparation

1 Prepare 100 mL of

Dulbecco's Phosphate Buffer Saline **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D8537

+2%

Fetal Bovine Serum, Premium **Gibco - Thermo Fisher Scientific Catalog #**A5670701

for two blood vials (~ 15 mL total) under the hood

2 Prepare 50 mL stock of Erylysis Buffer as follows: EDTA [M] 0.1 millimolar (mM) , NH_4Cl [M] 155 millimolar (mM) , $KHCO_3$ [M] 10 millimolar (mM) . Adjust the pH to 7.3 - 7.4 with HCl or NaOH. Erylysis buffer can be stored long term at 4 °C

3 Bring Lymphoprep™ 500 mL **STEMCELL Technologies Inc. Catalog #**7851 ,

Dulbecco's Phosphate Buffer Saline **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D8537

+2%

Fetal Bovine Serum, Premium **Gibco - Thermo Fisher Scientific Catalog #**A5670701

solution, Erylysis buffer,

CryoStor CS10-100ml **STEMCELL Technologies Inc. Catalog #**07930 and

Mr. Frosty at room temperature (18 °C - 25 °C)

4 Add 15 mL

Lymphoprep™ 500 mL **STEMCELL Technologies Inc. Catalog #**7851

to the SepMate-50 **STEMCELL Technologies Inc. Catalog #**85450 using a 25 mL pipette by carefully pipetting it through the central hole of the SepMate-50 **STEMCELL Technologies Inc. Catalog #**85450 insert (plastic insert inside the tube).

Note

If needed, use a sterile (non-filter tip) 1000 µL pipette tip placed into the insert hole as a way to stabilize and guide the strip pipette and Lymphoprep solution through the hole.

PBMC isolation

5 Add 15 mL - 17 mL blood into a 50ml Falcon tubes **Corning Catalog #**352070 . Add equal volume of Dulbecco's Phosphate Buffer Saline **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D8537
+2%
Fetal Bovine Serum, Premium **Gibco - Thermo Fisher Scientific Catalog #**A5670701
to the 50ml Falcon tubes **Corning Catalog #**352070 . Close the lid and mix gently by inverting 2 times.

6 Keeping the SepMate-50 **STEMCELL Technologies Inc. Catalog #**85450 vertical, add the diluted blood sample by slowly pipetting it down the side of the tube. The sample will mix with the
density gradient medium above the insert. Close the lid.

7 Centrifuge the SepMate-50 **STEMCELL Technologies Inc. Catalog #**85450 at 1200xg for 00:10:00 at Room temperature with the brakes on (medium deceleration).

8 With care, remove the SepMate-50 **STEMCELL Technologies Inc. Catalog #**85450 lid. To reduce platelet contamination, pipette off and discard about 10 mL of supernatant from the surface using a 10 mL pipette – well above the enriched mononuclear cell layer.

9 Pour the remaining top layer containing the enriched mononuclear cells into a new 50ml Falcon tubes **Corning Catalog #**352070 by inverting the SepMate-50 **STEMCELL Technologies Inc. Catalog #**85450 to 180 degrees. To avoid pouring the red blood cells (RBCs), do not hold the SepMate-50 **STEMCELL Technologies Inc. Catalog #**85450 in the inverted position for longer than 00:00:02

10 Top up the enriched mononuclear cells in tube with Dulbecco's Phosphate Buffer Saline **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D8537
+2%
Fetal Bovine Serum, Premium **Gibco - Thermo Fisher Scientific Catalog #**A5670701
to 50 mL and centrifuge at 300xg for 00:05:00 to pellet PBMCs

11 Carefully discard the supernatant using a vacuum aspirator without disturbing the PBMC pellet

12 To get rid of residual red blood cells (RBCs), resuspend the pellet in 5 mL - 7 mL of Erylysis Buffer and incubate at Room temperature for 00:05:00

13 Top up the remaining enriched mononuclear cells with Dulbecco's Phosphate Buffer Saline **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D8537
+2%
Fetal Bovine Serum, Premium **Gibco - Thermo Fisher Scientific Catalog #**A5670701
in 50ml Falcon tubes **Corning Catalog #**352070 and centrifuge at 300xg for 00:05:00 to
pellet PBMCs

14 Carefully discard the supernatant using a vacuum aspirator without disturbing the PBMC pellet

15 Resuspend the PBMC pellet in 3 mL - 4 mL of
CryoStor CS10-100ml **STEMCELL Technologies Inc. Catalog #**07930 or
Fetal Bovine Serum, Premium **Gibco - Thermo Fisher Scientific Catalog #**A5670701
+10% DMSO **Panreac AppliChem Catalog #**A3672

16 Count the cell suspension manually or using an automated cell counter . Adjust the volume of freezing media to
reach the desired concentration of PBMCs (1 - 20*10^6 cells/mL)

17 Prepare 0.5 mL - 1 mL aliquots in
CRYO.S 2 ML PP ROUND BOTTOM INTERNALTHREAD **greiner bio-one Catalog #**122263-2DG
and place them in Mr. Frosty. Cryopreserved PBMCs should be transferred into liquid nitrogen for long term storage after at least 24 hours at -80 °C . If cells are being used for experimentation within a few weeks of harvesting, they can be kept at -80 °C .

Documentation

18 Document the following information:
ID number and date
Time the blood was received. Time at first centrifugation step. Time the PBMCs were frozen. Time/date of transfer to liquid nitrogen.
Cell number, concentration and number of cryovials.

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