

Jun 09, 2018 Version 4

PBMC Isolation from apheresis collars with SepMate tubes V.4

DOI

dx.doi.org/10.17504/protocols.io.qpcdviw

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DOI: dx.doi.org/10.17504/protocols.io.qpcdviw

Protocol Citation: Girija Goyal 2018. PBMC Isolation from apheresis collars with SepMate tubes. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.qpcdviw>

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Protocol status: Working

We use this protocol and it's working

Created: June 02, 2018

Last Modified: June 09, 2018

Protocol Integer ID: 12740

Keywords: PBMC Isolation

Abstract

Commonly used protocol to isolate peripheral blood mononuclear cells from whole human blood or apheresis packs

Guidelines

Objective: Isolate peripheral blood mononuclear cells from fresh whole blood or apheresis packs, also referred to as Leukopaks or collars. In our case, these are platelet-depleted samples of human blood given from a donor. These can vary in volume and cell composition.

Materials

STEP MATERIALS

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

⊗ Lymphoprep™ 250 mL **STEMCELL Technologies Inc. Catalog #7801**

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Safety warnings

❗ Any materials that come into contact with blood should be sterilized with 10% bleach before discarding








Before start

- Make sure to repeatedly label sample with donor number, especially if working with multiple donors
- The protocol here is optimized for 10ml of material from platelet apheresis collars. Variations for other sources have been described.



- 1 Cut cone from collar and place on 50mL conical tube. Cut the top end to release blood and allow it to drip into the tube
- 2 Dilute blood collected from apheresis collar with equal volume RPMI or PBS. Mix well.

Isolation of PBMCs

- 3 Use a 50ml SepMate tube and follow manufacturer's directions for density centrifugation
 -  SepMate™-50 (IVD) 100 Tubes **STEMCELL Technologies Inc. Catalog #85450**
 -  Lymphoprep™ 250 mL **STEMCELL Technologies Inc. Catalog #7801**
- 4 Collect the white layer of cells just above the plastic insert into a final volume of 20 ml of media or buffer. Limit the amount of Lymphoprep that is harvested. Mix well.
- 5 Centrifuge at 300 g for 5 minutes.
 -  00:05:00 Centrifugation
- 6 Aspirate media and resuspend cells in 20 mL media/buffer depending on next steps.
 -  20 mL Warm media
- 7 Dilute 100x by adding 10ul cell solutions to 890 ul media/buffer and 100ul of Trypan blue
 -  10 µL Cell solution
 -  890 µL Media/buffer
 -  100 µL Trypan blue

Cell counting

- 8 Count cells using a hemocytometer. Count the number of cells in each of the four quadrants. Use the following formula to find the total number of cells.

$$\text{Cells/ml} = \text{cells counted} * \text{dilution factor} * 10^4 \text{ cells} / 4$$
- 9 Cells can be kept in solution in the refrigerator for up to two hours.