VERSION 4

JUN 09, 2018

OPEN ACCESS

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>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Jun 02, 2018

Last Modified: Jun 09, 2018

PROTOCOL integer ID:
 12740

Keywords: PBMC Isolation

PBMC Isolation from apheresis collars with SepMate tubes V.4

Girija Goyal¹

¹Wyss Institute, Harvard University

Girija Goyal

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

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

Lymphoprep™ 250 mL STEMCELL Technologies Inc. Catalog #7801

SAFETY WARNINGS

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

BEFORE START INSTRUCTIONS

- 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

- 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.

$\text{Cells/ml} = \text{cells counted} \times \text{dilution factor} \times 10^4 \text{ cells/4}$

- Cells can be kept in solution in the refrigerator for up to two hours.