PBMC Isolation from apheresis collars with SepMate tubes

Version 4

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Abstract

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

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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.

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.

Protocol

Step 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

Step 2.

Dilute blood collected from apheresis collar with equal volume RPMI or PBS. Mix well.

Isolation of PBMCs

Step 3.

Use a 50ml SepMate tube and follow manufacturer's directions for density centrifugation

REAGENTS

SepMate™-50 (IVD) 100 Tubes
85450 by Stemcell Technologies

Lymphoprep™ 250 mL 7801 by Stemcell Technologies

Step 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.

Step 5.
Centrifuge at 300 g for 5 minutes.

Step 6.
Aspirate media and resuspend cells in 20 mL media/buffer depending on next steps.

AMOUNT
20 ml Additional info:
Warm media

Step 7.
Dilute 100x by adding 10ul cell solutions to 890 ul media/buffer and 100ul of Trypan blue

AMOUNT
10 µl Additional info: Cell solution
AMOUNT
890 µl Additional info: Media/buffer
AMOUNT
100 µl Additional info: Trypan blue

Cell counting

Step 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.

Cells/ml = cells counted*dilution factor*104cells/4

Step 9.
Cells can be kept in solution in the refrigerator for up to two hours.

Warnings

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

discarding