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PBMC Isolation from apheresis collars with SepMate tubes V.4

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

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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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Troubleshooting

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.

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

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