PBMC Isolation Version 2

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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.
Acquire blood sample from hospital (in our case, from Brigham & Women's Hospital blood donor center)

Step 2.
Cut collar and drain blood into 50mL conical tube.

Step 3.
Dilute Leukopak with equal volume RPMI or PBS. Mix well. Whole blood does not need to be diluted.

Step 4.
Slowly layer solution on top of 10 mL density gradient solution.

Step 5.

Centrifuge at 300 g for 20 minutes at room temperature. Set acceleration and deceleration levels to minimal.

TEMPERATURE

22 °C Additional info:

Step 6.
Remove white layer of PBMCs using a 5 mL pipette tip.

Step 7.
Add these cells to 10 mL warm media in a 50 mL tube.

Step 8.
If using 5 ml or more of the Leukopak, you may have a very high number of cells. To effectively wash them, fill tube to 50 mL.

Step 9.
Centrifuge at 300 g for 5 minutes. Return acceleration / deceleration levels to high or 9.

Step 10.
Aspirate media and resuspend cells in 20 mL warm media per 10 ml of starting Leukopak. Steps 10-12 can be optimized depending on your yield.

Step 11.
For our starting material, dilute cells serially to 1000x. First dilute 100x by adding 10ul cell solutions to 990 ul media in a 1ml eppendorf tube. Then add 10 ul of the 100x dilution to 80 ul media. Add 10 ul trypan blue to this solution.

Step 12.
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.total # of cells = cells counted 4dilution factor 104cellsmltotal volume (ml)

Step 13.
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