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## PBMC Isolation V.2

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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.

## 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 Acquire blood sample from hospital (in our case, from Brigham & Women's Hospital blood donor center)
- 2 Cut collar and drain blood into 50mL conical tube.
- 3 Dilute Leukopak with equal volume RPMI or PBS. Mix well. Whole blood does not need to be diluted.
- 4 Slowly layer solution on top of 10 mL density gradient solution.
- 5 Centrifuge at 300 g for 20 minutes at room temperature. Set acceleration and deceleration levels to minimal.

 22 °C
- 6 Remove white layer of PBMCs using a 5 mL pipette tip.
- 7 Add these cells to 10 mL warm media in a 50 mL tube.
- 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.
- 9 Centrifuge at 300 g for 5 minutes. Return acceleration / deceleration levels to high or 9.
- 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.
- 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.
- 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.
$$\text{total \# of cells} = \frac{\text{cells counted} \times \text{dilution factor}}{\text{total volume (ml)}}$$
- 13 Cells can be kept in solution in the refrigerator for up to two hours.

