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PBMC Isolation from apheresis collars

Forked from [PBMC Isolation](#)

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1 *Works for me* [dx.doi.org/10.17504/protocols.io.56bg9an](https://doi.org/10.17504/protocols.io.56bg9an)



ABSTRACT

Commonly used protocol to isolate peripheral blood mononuclear cells from whole human blood modified here for apheresis collars

GUIDELINES

Objective: Isolate peripheral blood mononuclear cells from platelet apheresis collars.

SAFETY WARNINGS

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

BEFORE STARTING

- 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 collar and make an incision to drain blood into 50mL conical tube.
- 2 Dilute blood product with 2X volume RPMI or PBS. Mix well.
- 3 Slowly layer solution on top of 10 mL density gradient solution.
- 4 Centrifuge at 300 g for 25 minutes at room temperature. Set acceleration and deceleration levels to minimal.
22 °C
- 5 Remove white layer of PBMCs using a 5 mL pipette tip.
- 6 Add these cells to 10 mL warm media in a 50 mL tube.
- 7 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.
- 8 Centrifuge at 120 g for 10 minutes to remove platelets and get an accurate count. Return acceleration / deceleration levels to high or 9.

- 9 Aspirate media and resuspend cells in 20 mL warm media per 10 ml of starting blood product. Steps 10-12 can be optimized depending on your yield.
- 10 Dilute 100x by adding 10ul cell solutions to 890 ul media in a 1ml eppendorf tube and 100ul Trypan blue
- 11 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. $\text{total \# of cells} = (\text{cells counted} / 4) \times \text{dilution factor} \times 10^4 \text{ cells/ml}$
- 12 Cells can be kept in solution in the refrigerator for up to two hours.



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