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ViriCan Protocol: PBMC isolation from whole blood

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protocol .

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Protocol for isolation of peripheral blood mononuclear cells for downstream applications requiring viable cells or cell pellets to extract DNA or RNA.

Deilson Elgui De Oliveira 2022. ViriCan Protocol: PBMC isolation from whole blood. **protocols.io** https://protocols.io/view/virican-protocol-pbmc-isolation-from-whole-blood-b83rrym6

blood, mononuclear cells, PBMC isolation

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In steps of
Total RNA extraction from cells in suspension or cell lysates with Trizol™

Notes:

1. From Thermofisher (Answer Id: E4470): *"Blood collected with EDTA typically has the highest DNA contamination, blood collected with heparin typically has less than that collected with EDTA, and blood collected with citrate shows the least DNA contamination of the three. (Formulation for citrate solution: 3.8% (w/v) which is 3.8 g/100 mL of water. Use 0.5 mL for every 4.5 mL of blood. Rock gently back and forth after adding citrate solution to mix.) Adding 12 µL of 5 N acetic acid per milliliter of TRIzol Reagent may help, although there may still be a problem with DNA contamination. Using plasma or serum works best. The fresher the blood sample the better the RNA. Degraded RNA has been observed in blood that has been processed in as little as two hours after drawing."*

Reagent manuals:

- **Ficoll-Paque Plus:** https://drive.google.com/file/d/1hlvoGTZ60NTTsKaTwFiznoF1eh4vufwm/
- **Histopaque 1077**: https://drive.google.com/file/d/1jgZXuhhGaw0cCRLKa9K_1W2KaOLgPhHq/

Recommended blood collection tubes :

Lavender cap, with EDTA (Ethylenediaminetetraacetic Acid)

Green cap, with Heparin (Sodium/Lithium/Ammonium)

PBMC Separation Reagents:

Ficoll Paque PLUS **Ge**

Healthcare Catalog #17144003-500 ml

Histopaque 1077 **Sigma**

Aldrich Catalog #1077-1

Blood colection

1 Collect **4 mL** of whole blood in tubes with anticoagulant*

(*) Collection tubes with EDTA (lavender cap) or heparin (green cap)

PBMC isolation

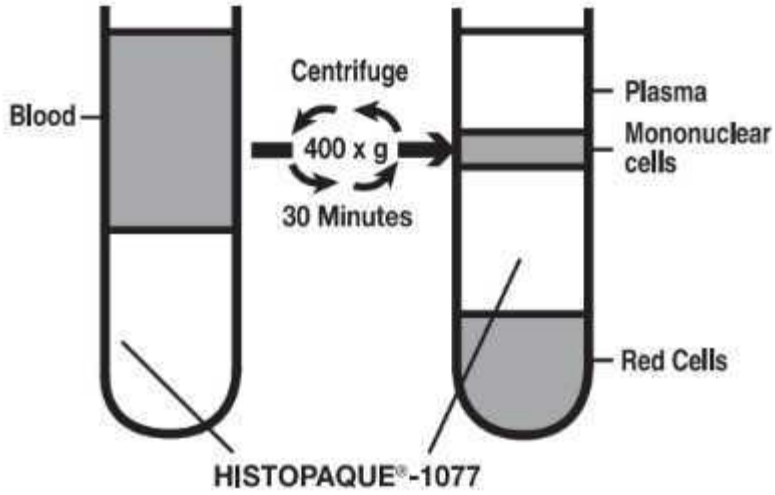
2 Add **3 mL** of Histopaque/Ficoll to 15mL conical centrifuge tube

Bring the tubes to **Room temperature** before use

3 Carefully layer about **3 mL** of whole blood on top of the Histopaque/Ficoll reagent.

Let the blood layer the separation reagent by pipetting it on the wall of the conical tube with a Pasteur pipette or 1mL pipette tip.

4 Centrifuge **Room temperature** at **400 x g, 00:30:00** ,



Centrifugation at lower temperatures (e.g., 4°C) may result in cell clumping and poor recovery.

5 Carefully transfer the middle opaque phase containing the PBMC to a new identified tube as follows and proceed to obtain PBMC's lisates or viable PBMCs for cellular assays.

For DNA/RNA extraction: nuclease-free 2mL microcentrifuge tube. Go to procedure starting in step 6
To isolate viable PBMC: 15mL centrifuge conical tubes. Go to the procedure starting Step 8

(Optional) PBMC lysis to extract DNA/RNA

6 Pellet the cells by centrifugation **1000 x g, 4°C, 00:05:00** 5m

Washing cells before addition of TRIZOL® Reagent should be avoided as this increases the possibility of mRNA degradation

6.1 Add To the PBMCs pellet:

250 µL of TRIZol LS Reagent

(General recommendation: **1 mL** of TRIZol per 5-10 ×10e6 eukaryotic cells)

6.2 ⚠

Homogenize to lysate the cells

NOTE: According to ThermoFisher, "*After homogenization and before the addition of chloroform, samples can be stored at -60 to -70°C for at least one month.*"

6.3 Store PBMC lisates in Trizol at **-70 °C (ULT Freezer)** or immediately proceed to nucleic acid isolation

RNA extraction with Trizol: https://www.protocols.io/view/total-rna-extraction-from-pbmc-with-trizol-reagent-b83trynn

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(Optional) PBMC clearing 10m

8 To the 15mL conical centrifuge tubes, add **10 mL** of **On ice** isotonic 1X PBS (Phosphate-buffered saline)

8.1 Centrifuge **250 x g, 00:10:00** 10m

8.2 Aspirate the supernatant and discard

8.3 Wash the PBMC pellet

8.4 Resuspend the cell pellet with **5.0 mL** isotonic 1X PBS (Phosphate-buffered saline)

8.5 Mix by gentle aspiration with a Pasteur pipet or pipetting up and down with 1mL tip

8.6 Centrifuge **250 x g, 00:10:00** 10m

8.7 Discard supernatant

8.8 Repeat the PBMC wash procedure twice

9 Proceed to downstream applications with the isolated PBMCs