Version 2a, Last updated 20 June 2023

ab241001 Whole Blood Polymorphonuclear Cell Isolation Kit

For the isolation of Polymorphonuclear cells from human whole blood.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Whole Blood Polymorphonuclear Cell Isolation Kit (ab241001) is unique in the market because it enables the user to isolate Polymorphonuclear cells (PMNs), assess viability of the obtained cell fractions and also determine purity with three simple, easy-to-use reagents. High yields, or 60-80% of total PMNs have been obtained yielding ≥ 5 x10^6 cells per mL of human whole blood. Purity of the cell fraction is assessed with giemsa stain (included). The kit's use results in highly enriched (>95%) cell fraction of polymorphonuclear cells with less than 3% red blood cell counts. More than 99% of the cells are viable, as determined by the Viability Stain.

2. Protocol Summary

Add whole blood to PMN Density Gradient Solution/PMN Density Gradient Media and centrifuge at 400 x *g* for 30 minutes at RT.

Remove plasma and PBMC layers with a needle.

Withdraw the PMN layer with a clean needle.

Add 6 mL of PMN Isolation Buffer. Centrifuge at 400 x *g* for 10 min and discard supernatant.

Prepare a 1:10 dilution of viability stain mix/viability stain. Determine the total cell count.

Illuminate the same region with light from the fluorescent lamp with the FITC/TRITC filter combination to count the fluorescent cells. Record the number of green and red cells.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at 4°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
PMN Isolation Buffer/PMN Isolation Buffer (1X)	2 x 30 mL	4°C
PMN Denstiy Gradient Solution/PMN Density Gradient Media	2 x 20 mL	4°C
Viability Stain Mix/Viability Stain	200 µL	4°C
Blunt-End Needle/Blunt-end needle 18 G; 1.5 in. (Sterile)	15 pieces	4°C
Giemsa Stain	7 mL	4°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 3 mL or 5 mL syringes
- Hemocytometer
- 50 mL conical tubes (polypropylene or polyethylene)
- dH_2O for removal of excess Giemsa
- Centrifuge with Swinging bucket rotor
- Brightfield Microscope, Fluorescent Microscope with FITC/TRITC Filters
- 50 and 1.5 mL sterile centrifuge tubes
- Human blood 30 mL collected in EDTA (final concentration 1.5-2.0 mM)

- Glass Slides to evaluate purity of cell fraction obtained

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 PMN Isolation Buffer/PMN Isolation Buffer (1X):

Ready to use as supplied. Store at 4°C. It should be opened and used in a sterile environment to prevent contamination. Bring to room temperature before use and mix well.

6.2 PMN Density Gradient Solution/PMN Density Gradient Media:

Ready to use as supplied. It should be opened and used in a sterile environment to prevent contamination. Bring to room temperature before use and mix well.

6.3 Viability Stain Mix/Viability Stain:

Ready to use as supplied. Store in the dark at 4°C.

6.4 Blunt-End Needle/Blunt-end needle 18 G; 1.5 in (Sterile):

Store at RT. The needle is not sharp, however it should be disposed of in an appropriate Sharps Collection & Disposal System.

6.5 Giemsa Stain:

Store in the dark at RT.

7. Sample Preparation

ΔNote: Work in a sterile environment. Use universal precautions when handling blood products and human body fluids.

7.1 Bring PMN Density Gradient Solution/PMN Density Gradient Media (PMN DGM) to RT and mix well by inverting bottle several times. Add 10 mL PMN DGM to a 50 mL centrifuge tube.

7.2 To add whole blood: hold the conical tube at a 45° angle, place tip of pipette at the edge of the PMN DGM and slowly add 10 mL whole blood onto the PMN DGM (see Figure 1). Carefully handle tube to prevent mixing of PMN DGM with blood. Centrifuge tube at 400 x *g* for 30 minutes at RT.

ΔNote: It is preferable to spin tubes in a swinging bucket rotor without brake.

7.3 Remove conical tube from centrifuge and observe the 6 layers (top to bottom): plasma, Peripheral Blood Mononuclear Cells (PBMCs), PMN DGM, PMNs, PMN DGM and RBCs at the bottom of the tube. It is normal/expected to see 2 layers of DGM.

7.3.1 PBMC removal: with an 18 G 1.5" needle affixed to 3 mL syringe withdraw plasma to within 1 mL (1 cm^3) of the PBMC layer. Discard plasma by expunging solution into waste container. With the same needle, withdraw the PBMC layer. Highest yields can be obtained by withdrawing approximately 1 mL above and 1 mL below the PBMC layer, which contains a small amount of plasma (above) or density gradient solution/density gradient media (below). Expunge PBMC layer into a clean 50 mL centrifuge tube if further use is desired.

7.3.2 Isolation of PMNs: with a clean needle, withdraw the PMN layer. Place the tip of the blunt end needle slightly above the PMN layer and circle the inside perimeter of the tube to extract the PMNs. The volume obtained is approximately 3 mL, expunge this solution with PMNs into a clean 50 mL centrifuge tube.

7.4 Add 6 mL of PMN Isolation Buffer/PMN Isolation Buffer (1X) to isolated PMNs. Centrifuge PMN suspension at 400 x *g* for 10 minutes and discard supernatant. Repeat wash, discard supernatant and resuspend in 5 mL of diluted PMN Isolation Buffer.

ΔNote: Observe cell pellet at bottom of tube. PMNs may also cling to side of tube. Do not disturb pellet when pouring off excess PMN Isolation Buffer.

8. Determination of Live Cell Count:

8.1 Prepare a 1:10 dilution of viability stain with cell suspension by adding 2 µL of Viability Stain Mix/Viability Stain to 18 µL of washed PMN suspension in a 1.5 mL centrifuge tube. Tap to mix. Inoculate hemocytometer with 10 µL of stained PBMC suspension.

8.2 Determine and record the total cell count with a Bright-field microscope.

8.3 With the same Region of Interest (ROI) in view, illuminate the slide with light from the fluorescent lamp with the FITC/TRITC filter combination to count the fluorescent cells. If a small amount of incidental white light illuminates the hemocytometer, the grid will be visible allowing the viewer to see the same ROI as visible with fluorescent light.

8.4 Live cells will fluoresce green. Dead cells will fluoresce red.

8.5 Tally the number of green and red cells to complete the calculations using the equations in Measurement section below.

9. Evaluation of Cell Suspension Purity

9.1 Remove 25 µL of PMN suspension and add to clean glass slide.

9.2 Allow the solution on slide to completely dry (15 min) or place on warming plate at 37°C to expedite drying process.

9.3 Add 50 µL of Giemsa Stain to stain cells and proceed with staining cells for 5 minutes. Wash slide until runoff is clear. Allow slide to dry.

9.4 Cells and morphology are best viewed with oil immersion objective lens.

9.5 Perform differential cell count using the formula described in 10.5 Measurement (below).

10. Measurement

10.1 Number of RBCs = Total Cell Count (hemocytometer) − Total Number of Fluorescent Cells (both red and green cells)

10.2 Percent Live Cells = Number of Green Fluorescent Cells/Total Number of Fluorescent Cells × 100

10.3 Percent Dead Cells = Number of Red Cells/Total Number of Fluorescent Cells × 100

10.4 Percent RBCs = Number of RBCs/Total Cell Count (hemocytometer) × 100

ΔNote: Contamination of PMNs with RBCs may affect downstream applications including such as flow cytometry or neutrophil chemotaxis assays. Therefore, if RBCs are >10% of total PBMC count we recommend that RBC lysis be completed.

10.5 Differential cell count: with a 40X objective, count a total of 200 cells. Observe the nucleus of each cell, and score those with multilobed nucleus as a PMN. Mononuclear cells or anucleate cells are scored as "not PMNs." Determine the percent of PMNs and not PMNs using the formula:

% PMNs = Number of Multilobed cells/Number of cells (200) x 100

11. Typical Data

Typical data provided **for demonstration purposes only**.

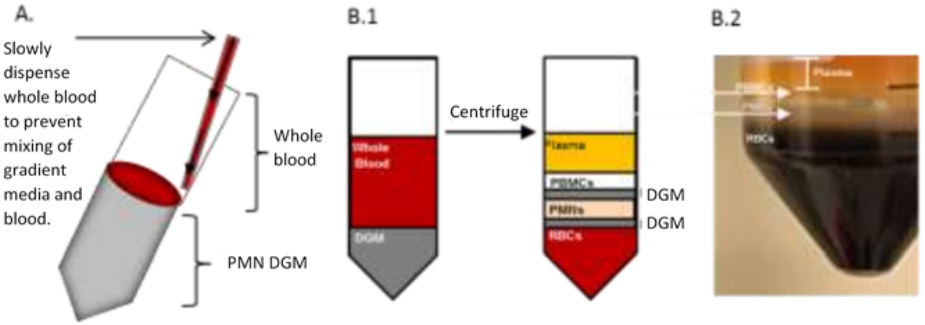

Figure 1. **A.** Illustration of conical tube held at 45 degrees while blood layered on top of PMN DGM. **B.1**. Layers of PMN DGM and whole blood prior to and after centrifugation showing the separation of layers in the conical tube. **B.2** Illustrates separation of six layers (plasma, PBMCs, PMN DGM, PMNs, PMN DGM and RBCs) obtained after spinning whole blood with PMN DGM.

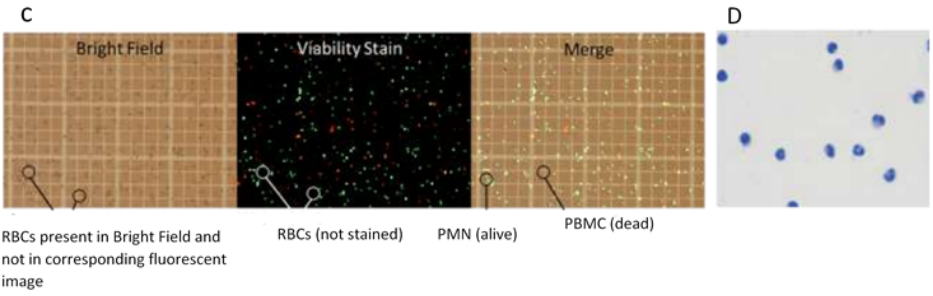

Figure 2. C. Bright-Field image of hemocytometer showing total cells(left); image from Fluorescent microscope with Rhodamine/FITC filters of same ROI showing live (green) and dead (red) cells (middle); merge of two panels (right). **D.** Polymorphonuclear Cells stained with Giemsa stain indicating purity of cell suspension obtained.

12. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)