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# ab234628 Human Peripheral Blood Mononuclear Cell Isolation and Viability Kit

For the isolation of PBMCs from human whole blood and determination of their viability and purity.

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

# Table of Contents

# 1. Overview

Human Peripheral Blood Mononuclear Cell Isolation and Viability Kit (ab234628) for PBMC Isolation is the first kit available to provide a complete set of components for the isolation of PBMCs from human blood and also allow visualization of viable cells. High yields of PBMCs (≥ 2.5 x$10^6$ cells/ml) have been obtained following this simple and time-saving protocol. By using the Viability Stain, it was proven that 99% of the cells are viable and the isolated fraction contains low red blood cell counts (≤ 3%).

Dilute 30 mL whole blood with an equal volume of RPMI 1640 media.

Layer diluted whole blood onto Density Gradient Media and centrifuge at 400 x *g* for 30 minutes at room temperature.

Remove plasma with needle and discard.

Remove PBMC layer with needle. Wash by centrifugation/resuspension with RPMI 1640 media.

Resuspend washed PBMCs in 2 mL RPMI 1640 media.

Determine cell count, viability and purity using Viability Stain.

## 2. Materials Supplied and Storage

Store kit at 4°C in the dark immediately on receipt and check below 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.

Avoid repeated freeze-thaws of reagents.

| Item | Quantity | Storage temperature (before prep) | Storage temperature (after prep) |
|---|---|---|---|
| Density Gradient Media | 60 mL | 4°C | 4°C |
| Viability Stain | 200 µL | 4°C | 4°C |
| EDTA (0.5 M, pH 8.0) | 0.5 mL | 4°C | 4°C |
| Blunt-end needle (18 G; 1.5 inch, sterile) | 10 units | Room temperature | N/A |

## 3. Materials Required, Not Supplied

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

- RPMI Media 1640, no phenol red.
- 5 mL syringes.
- Hemocytometer.
- 15 or 50 mL conical tubes (polypropylene or polyethylene).
- Swinging bucket rotor.
- 1.5 mL centrifuge tubes.
- Fluorescent Microscope with dual band bandpass excitation and emission filters capable of simultaneous FITC/TRITC detection.

## 4. 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.

# 5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

## 5.1 Density Gradient Media

1. Ready to use as supplied.
2. Open and use in a sterile environment to prevent contamination.
3. Bring to room temperature and mix well before use.

## 5.2 Viability Stain

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

## 5.3 EDTA (0.5 M, pH 8.0)

1. Ready to use as supplied.
2. Open and use in a sterile environment to prevent contamination.
3. Bring to room temperature and mix well before use.

## 5.4 Blunt-end needle (18 G, 1.5 inch, sterile)

1. Ready to use as supplied.

# 6. Isolation Procedure

**ΔNote:** Work in a sterile environment.

**ΔNote:** Use universal precautions when handling blood products and human body fluids.

## 6.1 PBMC preparation:

1. Dilute 30 mL whole blood 1:1 in RPMI Media 1640 (RPMI). Add 120 µL of 0.5 M EDTA to reach a final concentration of 1 mM EDTA. Invert to mix.

   **ΔNote:** Do not shake or vortex. Thirty mL of whole blood diluted 1:1 with RPMI yields 60 mL diluted blood.

2. Bring Density Gradient Media to room temperature and invert to mix. Add 20 mL Density Gradient Media to a sterile 50 mL conical tube. Hold the conical tube at a 45° angle, then place the pipette tip at the edge of the angled Density Gradient Media layer and slowly add 20 mL of diluted blood onto the Density Gradient Media (see Figure 1). Carefully handle centrifuge tubes with blood to prevent mixing of Density Gradient Media with diluted blood. Centrifuge tube at 400 x *g* for 30 minutes at room temperature.

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

3. Remove conical tube from centrifuge and observe the 4 layers (top to bottom): plasma, PBMCs, Density Gradient Media and RBCs. With an 18 G 1.5" needle affixed to 5 mL syringe (not provided), carefully withdraw plasma to within 1 mL of the buffy coat. Discard plasma by expunging solution into waste container. With the same needle, withdraw the PBMC layer. To obtain the highest yield of PBMCs, it is best to include 1 mL above and 1 mL below the PBMC layer, which contains a small amount of plasma (above) or density gradient media (below). Expunge PBMC layer into a clean 50 mL falcon tube. This step may yield 3-7 mL of fluid.

4. Wash PBMCs. Add 10 mL RPMI media to the solution obtained in Step 6.1.3 (above). Centrifuge (250 x *g*, 10 minutes, room temperature) and discard excess media. Repeat wash 2X to remove plasma, platelets and Density Gradient Media from PBMCs. Carefully resuspend cells in 2 mL of RPMI in a 50 mL centrifuge tube. Proceed to Step 6.2.

ΔNote: Observe cell pellet at bottom of tube. PBMCs may also cling to side of tube. Do not disturb pellet when pouring off excess RPMI.

## 6.2 Determination of live cell count:

5. Prepare a 1:10 dilution of Viability Stain with cell suspension by adding 2 µL of Viability Stain to 18 µL of washed PBMC suspension in a 1.5 mL centrifuge tube. Carefully resuspend cell pellet. Inoculate hemocytometer with 10 µL of stained PBMC suspension. Determine and record the total cell count with a Bright-field microscope.
6. 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. Live cells will fluoresce green. Dead cells will fluoresce red. Tally the number of green and red cells to complete the calculations using the equations below (Section 7).

## 7. Data Analysis

1. Number of RBCs = Total cell count (hemocytometer) – Total number of fluorescent cells.
2. Live cells fractions = Number of green fluorescent cells/Total number of fluorescent cells x 100
3. Dead cells fraction = Number of red cells/Total number of fluorescent cells x 100
4. Percentage RBCs = Number of RBSs/Total cell count (hemocytometer) x100

**ΔNote:** Contamination of PBMCs with RBCs may affect downstream applications including, but not limited to flow cytometry or T Cell Killing Assays. For that reason, we recommend repeating the separation with remaining density gradient media if RBCs are >10% of total cells count.

## 8. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

## 9. Typical Data

Data provided **for demonstration purposes only.**

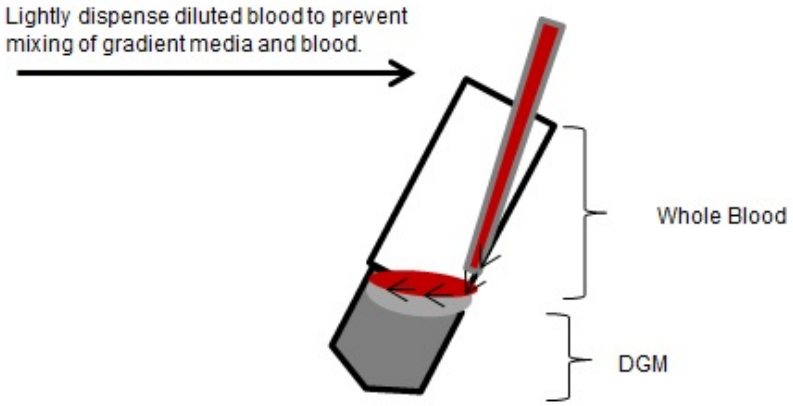


**Figure 1.** Illustration of conical tube held at an appropriate angle while blood is layered on top of Density Gradient Media.

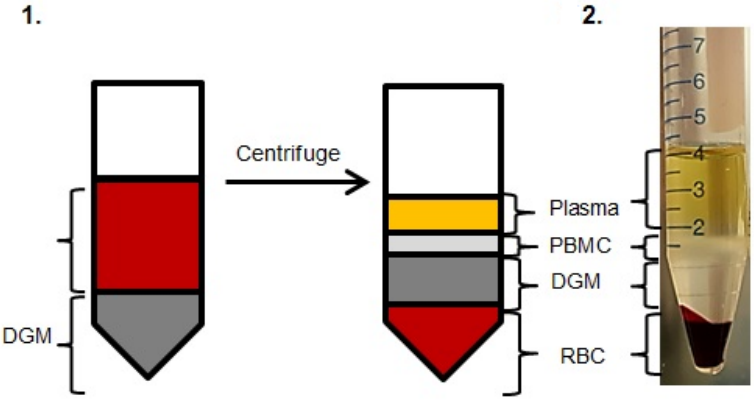


**Figure 2.** (1) Layers of Density Gradient Media and whole blood prior to and after centrigugation showing the separation of layers in the conical tube. (2) Illustrates the separation of four layers (plasma, PBMCs, Density Gradient Media and RBCs).

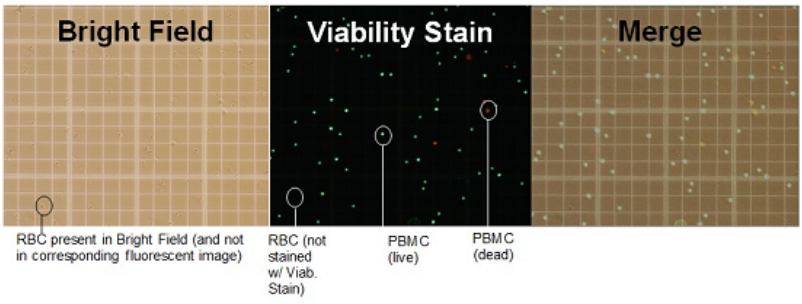


**Figure 3.** Brightfield image of hemocytometer showing total cells (left), image from fluorescent microscope with Rhodamine/FITC filters of the same ROI showing live (green) and dead (red) cells (middle). The two images are merged (right).

# 10. Notes

## Technical Support



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