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Human Peripheral Blood Mononuclear Cell Isolation and Viability Kit

(Catalog # K541-30; 30 ml PBMC isolation from whole blood; Store at 4°C)

I. Introduction:

Peripheral blood mononuclear cells (PBMCs) are found in the peripheral blood circulating throughout the body and not sequestered in the lymphatic system, spleen, liver or bone marrow. PBMCs are composed of four cell types: T cells, B cells, Natural Killer cells and monocytes, each having a round nucleus. Applicable research areas include: stem cell research, evaluation the immunotherapeutic potential of T or B cells and evaluation of inflammatory response orchestrated by cytokines and chemokines. Based on the unique density of each component, the whole blood is separated into plasma, PBMCs, density gradient media and red blood cells (RBCs). BioVision's Human Peripheral Blood Mononuclear Cell Isolation and Viability Kit 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 x10^6 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%).

II. Applications:

- Isolation of PBMCs from human whole blood.
- Determination of cell viability and also contamination from RBCs in isolated cell fraction.

III. Sample Types:

- Human whole blood (collected within 12 hr)

IV. Kit Contents:

Components	K541-30	Cap Code	Part Number
Density Gradient Media	60 ml	NM	K541-30-1
Viability Stain	200 µl	Red	K541-30-2
EDTA 0.5 M, pH 8.0	0.5 ml	Amber	K541-30-3
Blunt-end needle 18 G; 1.5 in. (Sterile)	10 pieces	-	K541-30-4

V. User Supplied Reagents and Equipment:

- 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

VI. Storage Conditions and Reagent Preparation:

Store kit at 4°C, protected from light. Briefly centrifuge vials prior to opening. Read entire protocol before performing the assay.

- **Density Gradient Media (DGM) and EDTA 0.5 M, pH 8.0** should be opened and used in a *sterile environment* to prevent contamination. Bring to room temperature before use and mix well.
- **Viability Stain** store in dark, at 4°C.
- **Blunt-end needle 18 G; 1.5" (Sterile)**: store at RT. The needle is not sharp, however should be disposed of in an appropriate Sharps Collection & Disposal System.

VII. PBMC Isolation and Viability Assay Protocol (10 ml or 100 ml total volume of whole blood):

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

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1. **Sample Preparation:**
   - **a.** 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. *Do not shake or vortex*. *Thirty ml of whole blood diluted 1:1 with RPMI yields 60 ml diluted blood.*
   - **b.** Bring Density Gradient Media (DGM) to RT 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 DGM layer and slowly add 20 ml of diluted blood onto the DGM (see Figure A). Carefully handle centrifuge tubes with blood to prevent mixing of DGM with diluted blood. Centrifuge tube at 400 *x g* for 30 minutes at RT.
     Note: It is preferable to spin tubes in a swinging bucket rotor without brake.
   - **c.** Remove conical tube from centrifuge and observe the 4 layers (top to bottom): plasma, PBMCs, DGM and RBCs. With an 18 G 1.5" needle affixed to 5 ml syringe (not provided), carefully withdraw plasma to within 1 ml (1 cm^3) 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*.
   - **d.** Wash PBMCs. Add 10 ml RPMI media to the solution obtained in step c (above). Centrifuge (250 x *g*, 10 minutes, RT), discard excess media. Repeat wash 2X to remove plasma, platelets and DGM from PBMCs. Carefully resuspend cells in 2 ml of RPMI in a 50 ml centrifuge tube. Proceed to Step 2. **Note:** Observe cell pellet at bottom of tube. PBMCs may also cling to side of tube. *Do not disturb pellet when pouring off excess RPMI.*
2. **Determination of Live Cell Count:**

a. 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.

b. 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.

3. Measurement:

a. $\text{Number of RBCs} = \text{Total Cell Count (hemocytometer)} - \text{Total Number of Fluorescent Cells}$

b. $\text{Live Cells Fraction} = \text{Number of Green Fluorescent Cells/Total Number of Fluorescent Cells} \times 100$

c. $\text{Dead Cells Fraction} = \text{Number of Red Cells/Total Number of Fluorescent Cells} \times 100$

d. $\text{Percentage RBCs} = \text{Number of RBCs/Total Cell Count (hemocytometer)} \times 100$

Note: 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.

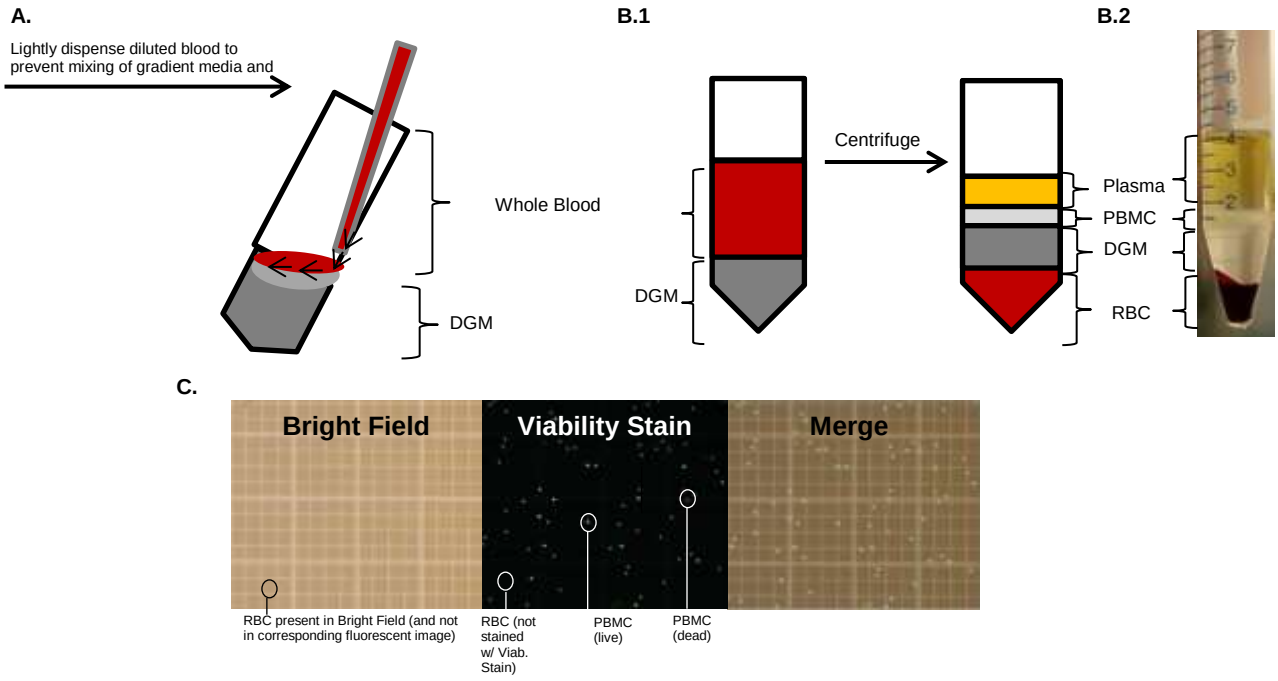

Figure A. Illustration of conical tube held at appropriate angle while blood layered on top of DGM. **Figure B.1.** Layers of DGM and whole blood prior to and after centrifugation showing the separation of layers in the conical tube. **Figure B.2** illustrates separation of four layers (plasma, PBMCs, DGM and RBCs). **Figure C**. Brightfield 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).

VIII. Related Products

1X Red Blood Cell Lysis Buffer (5830)
Propidium Iodide (1056)
10X Red Blood Cell Lysis Buffer (5831)

FOR RESEARCH USE ONLY! Not to be used on humans.