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Human Whole Blood Monocyte Isolation Kit

12/17

(Catalog # K933-30; monocyte isolation from 30 ml whole blood; Store at 4°C)

I. Introduction:

Monocytes are a type of white blood cell (leukocyte) and the largest of the immune cells. They are produced in the bone marrow and then migrate to the blood stream where they comprise 3-8% of all leukocytes. Depending on the clincial presentation of disease, monocytes will migrate to a specific tissue and then differentiate into a tissue-specific dendritic cell or a macrophage. Monocytes are easily identified by the large amoeboid shape, unilobar nucleus and vacuolated cytoplasm. They serve three important roles: phagocytosis, presentation of antigen to T cells which subsequently results in T cell clonal expansion, and the production of cytokines. BioVision's human Whole Blood Monocyte Isolation Kit allows for *in vitro* isolation of intact, viable monocytes. The kit enables the isolation of ≥ 5 x10^6 monocytes/ml yielding approximately 80% of the total monocytes present in 1 ml of whole blood. The viability stain (included) used to identify living monocytes indicates that more than 99% of the cells in the isolated fraction are viable. The isolated fraction is pure - it contains more than 95% monocytes with less than 3% red blood cell counts.

II. Applications:

- Isolation of monocytes from human whole blood.
- Determination of cell viability, nuclear morphology, purity, and also contamination from RBCs in the isolated cell fraction.
- Monocyte differentiation (into dendritic cells or macrophages) and characterization of cellular morphology.
- *In vitro* assays to evaluate primary monocyte functions such as phagocytosis, adhesion and chemotaxis.
- Monocyte activation testing as a functional readout of presence/absence of gram-positive or gram-negative organisms.

III. Sample Types:

- Fresh whole blood collected less than 12 hours prior to monocyte isolation.

IV. Kit Contents:

Components	K933-30	Cap Code	Part Number
Monocyte Isolation Buffer (MIB)	115 ml	NM	K933-30-1
Monocyte Density Gradient Media (MDGM)	25 ml	NM	K933-30-2
54% Density Media	15 ml	NM	K933-30-3
Viability Stain	200 µl	Red	K933-30-4
Blunt-end needle 18 G; 1.5 in. (Sterile)	10 pieces	-	K933-30-5
Giemsa Stain	7 ml	Amber/NM	K933-30-6

V. User Supplied Reagents and Equipment:

- 3 ml or 5 ml syringes
- RPMI 1640 Medium, no phenol red, 10% FCS ("RPMI w/serum")
- 1.5, 15 or 50 ml conical tubes (polypropylene or polyethylene)
- Centrifuge with Swinging bucket Rotor
- Fluorescent Microscope with a dual FITC/TRITC Filter
- Hemocytometer
- Human blood 30 ml collected in 1.5-2.0 mM EDTA
- Glass slides
- dH_2O

VI. Storage Conditions and Reagent Preparation:

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

- **Monocyte Isolation Buffer (MIB), Monocyte Density Gradient Media (MDGM), and 54% Density Media:** Store at 4°C and use in a *sterile environment* to prevent contamination of isolated monocytes. Bring to room temperature (RT) before use and mix well.
- **Viability Stain** store in dark, at 4°C.
- **Blunt-end needle 18 G; 1.5 in. (Sterile)**: store at room temperature. The needle is not sharp, however it should be disposed of in an appropriate Sharps Collection & Disposal System.
- **Giemsa Stain**: store with other kit components at 4°C or in dark. Mix well to ensure the solution is homogeneous before use. Bring to RT before use.

VII. Monocyte Isolation and Viability Assay Protocol (30 ml total volume of whole blood):

Work in a sterile environment. Use universal precautions when handling blood products and human body fluids. This kit has sufficient reagents to isolate monocytes from 30 ml of whole blood from one patient, or 10 ml samples from three patients.

1. Sample Preparation:

Prepare the following solutions to create the gradient:

a. RPMI w/serum: Combine 45 ml of RPMI 1640 Medium, no phenol red, with 5 ml FCS. Media without phenol red is preferable because it allows the user to see the opacities of the gradient associated with the density of each solution without the influence of color from phenol red.

b. Density Barrier Solution (DBS, 1.072 g/ml). Add 6.1 ml MDGM to 23.9 ml of RPMI w/serum. Mix well by inverting mixture several times.

c. Dilute Blood: Add 2 ml of 54% Density Media (included) per 10 ml of whole blood. Gently invert three times to mix.

Create the density gradient:

d. Add 4 ml of Dilute Blood to a 15 ml conical tube. Then, carefully layer 6 ml of Density Barrier Solution (**1.b**) over Dilute Blood. Add 0.5 ml RPMI w/serum on top of Density Barrier Solution **(See Figure A).**
Note: For each 10 ml sample of whole blood, there will be 12 ml of Dilute Blood. The 12 ml will be divided amongst 3 x 15 ml conical tubes. The monocytes isolated from each of these tubes can be pooled in **Step g** (below).

e. Centrifuge at 700 x g for 30 minutes at 4°C. *(Cold temperature prevents platelet aggregation and activation of monocytes)*, **(See Figure B).**

Note: It is preferable to centrifuge tubes in a centrifuge with swing-bucket rotor and no brake to prevent disturbance of pellet.

f. Remove conical tube and observe 4 layers (top to bottom): monocytes, Density Barrier Solution, plasma and RBCs. Collect monocytes that float to the top of the solution. There will be a slight gradient (or change in opacity) in the upper 2-3 ml of the top layer because of the monocytes distributed at the top **(Figure C).** Remove the top 2 ml (containing the monocytes) from each of the three tubes and suspend in a clean 50 ml conical tube. If cells are from the same patient, they can be pooled during the washes.

g. Dilute the collected monocytes with 18 ml of MIB. Centrifuge cells at 700 x g for 10 minutes, 4°C. Repeat twice and resuspend the pellet in 5 ml of complete media.

2. **Determination of Live Cell Count:**

a. Prepare a 1:10 dilution of Viability Stain with cell suspension by adding 18 µl of washed monocyte suspension and 2 µl of Viability Stain to a 1.5 ml centrifuge tube. Inoculate hemocytometer with 10 µl of stained monocyte suspension. Determine and record the total cell count with a Bright-field microscope.

b. With the same Region of Interest (ROI) in view, reduce white light, open fluorescent lamp shutter and view cells with a FITC/TRITC filter 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 was 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. **Evaluation of Purity in Cell Suspension:**

a. Remove 25 µl of monocyte suspension and add to a clean glass slide. Allow the solution on the slide to completely dry (15 min) or heat slide at 37°C to expedite the drying process. Add 50 µl of Giemsa Stain to dried cells and incubate with stain for 5 minutes. Wash slide until runoff is clear. Allow slide to dry. Cells and morphology are best viewed with oil immersion objective lens **(Figure D).** Perform differential cell count using the formulas described in **4. Measurement** (below).

4. **Measurement:**

a. $\text{RBCs}, \% = \{(\text{Total Cells (hemocytometer)} - \text{Number of Fluorescent Cells (red \& green)})/\text{Total Cells (hemocytometer)}\} \times 100$

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

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

Note: Contamination of monocytes with RBCs may affect downstream applications including, but not limited to flow cytometry, chemotaxis assays or cell differentiation protocols. For that reason, we recommend repeating the separation with remaining MDGM if RBCs are >10% of total PBMC count. Alternatively, RBC lysis buffer (Catalog #5830) can be used.

d. Differential Cell Count: with a 40X objective, count a total of 200 cells. Observe the nucleus of each cell, and score those with unilobar nucleus and vacuolated cytoplasm as a monocyte. Multinucleate cells or those with lobed nuclei are scored as "not monocytes." Monocytes, % =(Number of large unilobar nucleus)/(Total Number of Cells (200))×100.

Note: platelets are a small, anucleate blood component and not included in the differential cell counts.

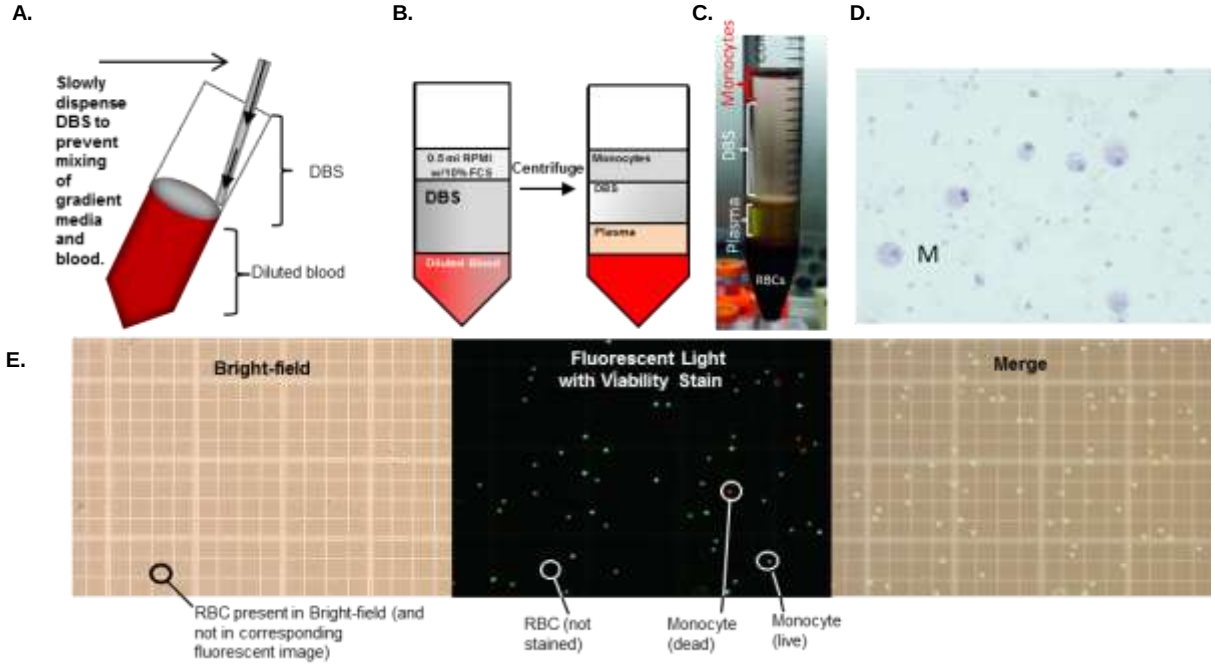

Figure A. Illustration of conical tube held at 45° angle while DBS is layered on top of Dilute Blood. **B.** Layers of DBS and whole blood prior to and after centrifugation showing the separation of layers in the conical tube. **C.** Separation of four layers (monocytes, DBS, plasma, and RBCs). **D**. Bright-field image of monocytes stained with Giemsa. The large, "fluffy" cytoplasm is approximately two-fold larger in size than the amoeboid nucleus (M, monocyte). **E.** Bright-field image of cells on hemocytometer to determine the Total Cell Count (Left), Image from Fluorescent microscope with FITC/TRITC filters of same ROI showing live (green) and dead (red) cells (middle); merge of two panels (right).

VIII. RELATED PRODUCTS:

Catalog # 5830 1X Red Blood Cell Lysis Buffer **Catalog # 1056** Propidium Iodide

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