ab303781 – Human Whole Blood Monocyte Isolation Kit

For Isolation of Monocytes from 30 mLs of human whole blood.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab303781

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 clinical 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. The human Whole Blood Monocyte Isolation Kit allows for in vitro isolation of intact, viable monocytes. The kit enables the isolation of ≥ 5 x106 monocytes/ml yielding approximately 80% of the total leucocytes 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.

Storage and Stability

The entire ELISA kit may be stored at 4°C, protected from light.

Materials Supplied

Item	Quantity
Monocyte Isolation Buffer (MIB)	115 ml
Monocyte Density Gradient Media (MDGM)	25 ml
54% Density Media	15 ml
Viability Stain	200 µl
Blunt-end needle 18 G; 1.5 in. (Sterile)	10 units
Giemsa Stain	7 ml

Materials Required, Not Supplied

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

- 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
- dH2O

Reagent Preparation

- 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.

Sample Preparation

Fresh Whole Blood: To be collected less than 12 hours prior to monocyte isolation

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

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.

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

Assay Procedure

- 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.

Create the density gradient:

- Add 4 ml of Dilute Blood to a 15 ml conical tube. Then, carefully layer 6 ml of Density Barrier Solution over Dilute Blood. Add 0.5 ml RPMI w/serum on top of Density Barrier Solution
    a. ***ΔNote:*** *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)*
- Centrifuge at 700 x g for 30 minutes at 4°C. (Cold temperature prevents platelet aggregation and activation of monocytes)
    a. ***ΔNote:*** *It is preferable to centrifuge tubes in a centrifuge with swing-bucket rotor and no brake to prevent disturbance of pellet.*
- 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 ayer because of the monocytes distributed at the top. 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.

- 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.

Determination of Live Cell Count:

- 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.
- 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.

Evaulation of Purity in Cell Suspension

- 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. Perform differential cell count using the formulas described in the calculations section (below)

Calculations

a) RBCs, % = {(Total Cells (hemocytometer) − Number of Fluorescent Cells (red & green))/Total Cells (hemocytometer)} x 100
b) Live Cells, % = Number of Green Fluorescent Cells/Total Number of Fluorescent Cells × 100
c) Dead Cells, % = Number of Red Cells/Total Number of Fluorescent Cells × 100
    a. *∆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, a RBC lysis buffer 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.
    a. *∆Note: platelets are a small, anucleate blood component and not included in the differential cell counts.*

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips: www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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