



Peripheral Blood Mononuclear Cell (human) Isolation Kit

Item No. 502891

www.caymanchem.com

Customer Service 800.364.9897

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a 4°C kit. After opening the kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage Temperature
10009322	Cell-Based Assay Buffer Tablet	1 vial/2 tablets	RT
601077	RBC Lysis Buffer (10X)	1 vial/10 ml	4°C
401153	Whole Blood Separation Histopaque® Reagent	1 vial/25 ml	4°C

NOTE: Histopaque® is a product of Sigma-Aldrich Co.

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Fresh (<4 hours after collection), whole, anticoagulated human blood
2. A source of pure water; glass distilled water or HPLC-grade water is acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
3. Filters (0.2 μ m) for sterile filtration of prepared reagents (typically syringe top or vacuum filtration devices)

INTRODUCTION

About This Kit

Cayman's Peripheral Blood Mononuclear Cell (human) Isolation Kit provides a quick and easy method for isolating peripheral blood mononuclear cells (PBMCs) from human whole blood. The method uses a density reagent, which cushions PBMCs above the interface, while neutrophils and red blood cells (RBCs) fall through. PBMCs isolated using this method are suitable for downstream studies, including monocyte differentiation, T cell activation, and phenotypic characterization by flow cytometry. This kit provides enough reagents to isolate PBMCs from 40 ml of human whole blood, typically yielding 15-40 million cells.

Reagent Preparation

1. Cell-Based Assay Buffer - (Item No. 10009322)

Dissolve one Cell-Based Assay Buffer Tablet in 100 ml of pure water. Filter through a 0.2 µm filter before using to dilute the whole blood. After filtration, this buffer is sterile, should be handled with aseptic technique, and will be stable for approximately one year at room temperature.

2. RBC Lysis Buffer (10X) - (Item No. 601077)

On the day of use, combine 5 ml of RBC Lysis Buffer (10X) with 45 ml of pure water to make RBC Lysis Buffer (1X). Warm to room temperature and sterile filter using a 0.2 µm filter prior to use. Discard any unused RBC Lysis Buffer (1X) after 48 hours. The remaining unused RBC Lysis Buffer (10X) will be stable for one year when stored at 4°C.

3. Whole Blood Separation Histopaque® Reagent - (Item No. 401153)

The vial contains 25 ml of Histopaque®. It is ready to use as supplied.

Sample Preparation

Whole Blood Preparation

1. Collect up to 40 ml of whole blood using an anticoagulant, such as heparin or EDTA.
2. Transfer the blood to 50 ml conical tubes, placing no more than 20 ml of whole blood into each tube.
3. Rinse the blood collection tubes with ½ volume of sterile-filtered Cell-Based Assay Buffer. Add the rinsed solution to the 50 ml conical tubes. For example, for 10 ml of whole blood collected into one collection tube, rinse the tube with 5 ml of Cell-Based Assay Buffer and combine with whole blood for a total volume of 15 ml.

Density Gradient Preparation

NOTE: Two methods to layer diluted blood over Histopaque® are available (see below) and either can be applied at the user's discretion.

1. **Overlayering method:** Pipet 10 ml of Whole Blood Separation Histopaque® Reagent into a sterile 50 ml conical tube. Slowly layer up to 30 ml of the diluted blood on the top of Whole Blood Separation Histopaque® Reagent, keeping the tip of the pipette at the top of the liquid to ensure minimal mixing of the Histopaque® with the blood.
2. **Underlayering Method:** Slowly pipet 10 ml of Whole Blood Separation Histopaque® Reagent underneath the 30 ml (maximum volume) of diluted blood in the conical tube, keeping the pipette tip well below the interface of the blood and Histopaque®. Take care to not push bubbles out of the pipette tip at the end of dispensing.

PBMC Isolation

1. Immediately after layering, centrifuge at 500 x g for 20-30 minutes at 18-26°C with the centrifuge brake set to low or off.
2. After removing the tube(s) from the centrifuge, it should resemble Figure 1 (see page 8). Carefully aspirate most of the yellowish top layer, leaving some above the fluffy white-gray PBMC layer. Carefully transfer the entire PBMC layer to a fresh, sterile 50 ml conical tube.
3. Add 25 ml of Cell-Based Assay Buffer to the cells, pipet gently to mix, and centrifuge at 400 x g for 5 minutes at 18-26°C.
4. Aspirate the supernatant (may be cloudy with platelets), and evaluate whether there is RBC contamination by observing whether the pellet is pink-to-red. If RBC contamination is present, resuspend the pellet in 10 ml of RBC Lysis Buffer (1X) and incubate for 2-5 minutes at 18-26°C. If RBC contamination is not present, skip to step 6.
5. Centrifuge at 400 x g for 5 minutes at 18-26°C and aspirate the supernatant.
6. Resuspend the cell pellet in 25 ml of Cell-Based Assay Buffer.
7. Centrifuge at 400 x g for 5 minutes at 18-26°C and aspirate the supernatant.

8. Resuspend the final pellet in approximately 10 ml of Cell-Based Assay Buffer. Cells can be used directly in downstream applications.

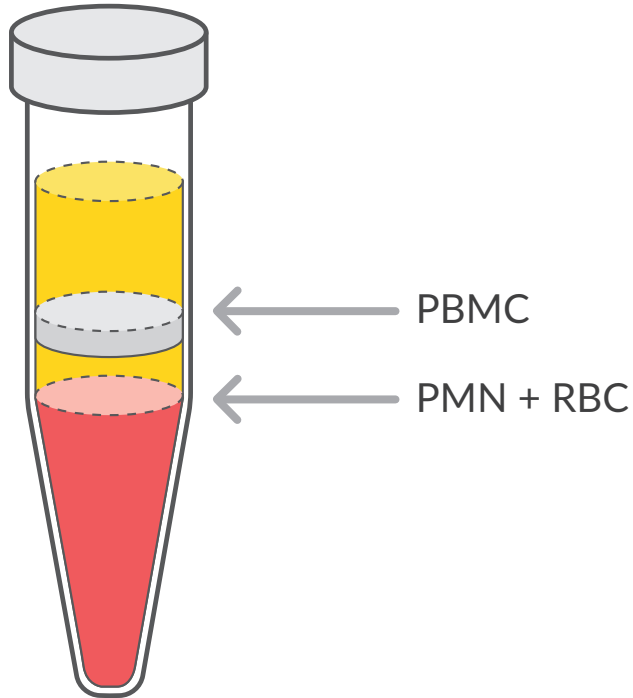


Figure 1. Graphical representation of layers after centrifugation. PBMCs are found in the fluffy white-gray layer between RBCs and serum.

ANALYSIS

Performance Characteristics

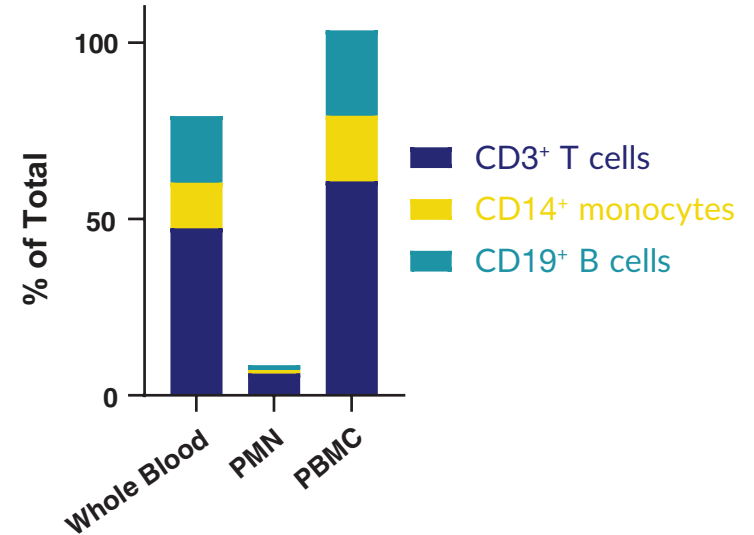


Figure 2. Enrichment of PBMC subsets obtained from PBMC (human) Isolation Kit. Whole blood (lysed with RBC lysis buffer), polymorphonuclear cells (PMNs), and PBMCs were stained with the indicated lineage marker antibodies and analyzed by flow cytometry.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Significant RBC contamination	A. Poor layering of blood over Histopaque® B. Recovery of RBC layer with PBMC layer	A. Try alternative technique for layering, centrifuge immediately B. Pipette the PBMC layer carefully, avoiding the RBC layer completely

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman’s Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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