Neutrophil (human) Isolation Kit

Item No. 502889

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

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
10009322	Cell-Based Assay Buffer Tablet	1 vial/1 tablet	RT
601077	RBC Lysis Buffer (10X)	1 vial/10 ml	4°C
600612	Cell-Based Assay Neutrophil Isolation Histopaque®	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).*

About This Assay

Cayman's Neutrophil (human) Isolation Kit provides a quick and easy method for isolating polymorphonuclear cells (PMNs) from human whole blood. The method utilizes a density reagent, which cushions peripheral blood mononuclear cells (PBMCs) above the interface, while PMNs and red blood cells (RBCs) fall through. PMNs isolated using this method are suitable for downstream studies, including phagocytosis, NETosis, and enzyme activity assays and flow cytometry. This kit provides enough reagents to isolate PMNs from 40 ml of human whole blood, typically yielding 25-50 million cells.

Reagent Preparation

NOTE: While it is recommended to prepare and use the following reagents in a biosafety cabinet, sterility may not be necessary for downstream applications.

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

   Dissolve one Cell-Based Assay Buffer Tablet in 100 ml of distilled water. Filter through a 0.2 µM filter before using to dilute the whole blood. This buffer should 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 distilled water to make RBC Lysis Buffer (1X). Warm to room temperature 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. **Cell-Based Assay Neutrophil Isolation Histopaque® - (Item No. 600612)**

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

Sample Preparation

Whole Blood Preparation

NOTE: While these isolation steps are typically performed in a biosafety cabinet, sterility may not be necessary for downstream applications, as neutrophils do not survive culture for more than 24 hours.

1. Collect up to 40 ml of whole blood using 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 rinse solution to the 50 ml conical tubes. For example, for 10 ml of whole blood collected into one collection tube, rinse tube with 5 ml 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 Cell-Based Assay Neutrophil Isolation Histopaque® into a clean 50 ml conical tube. Slowly layer up to 30 ml of the diluted blood on the top of Cell-Based Assay Neutrophil Isolation Histopaque®, 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 Cell-Based Assay Neutrophil Isolation Histopaque® 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.

Neutrophil 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 tube from centrifuge, it should resemble Figure 1 (see page 11). Carefully aspirate the yellowish and clear top layers and leave the deep red lower layer containing PMNs and RBCs in the tube.
3. Pipette 25 ml of RBC Lysis Buffer (1X) into the tube and mix well. Rock the tube on a rocker for 5-12 minutes to lyse the RBCs.
4. Centrifuge at 400 x g for 5 minutes at room temperature to pellet the PMNs.
5. Carefully aspirate the deep red supernatant, leaving approximately 1 ml at the bottom of the tube to ensure that the PMNs are not aspirated.
6. Add 5 ml of RBC Lysis Buffer (1X) to complete lysis of remaining RBCs, pipetting up and down to ensure complete suspension of the cell pellet. Incubate for 2-3 minutes at room temperature.
7. Centrifuge at 300 x g for 5 minutes at room temperature to pellet the PMNs.
8. Resuspend the cells in 20 ml of Cell-Based Assay Buffer. The cells can be washed with Cell-Based Assay Buffer once more or be utilized directly in downstream applications.

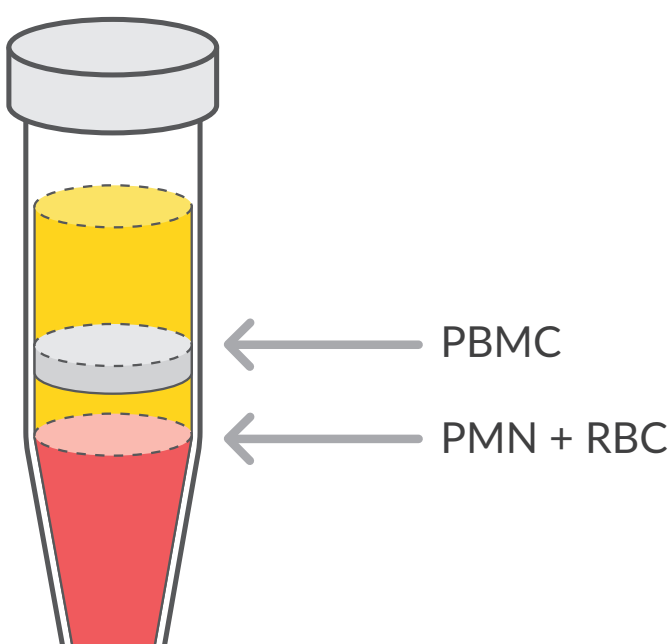

Figure 1. Graphical representation of layers after centrifugation. PMNs are found in the bottom layer with red blood cells.

Performance Characteristics

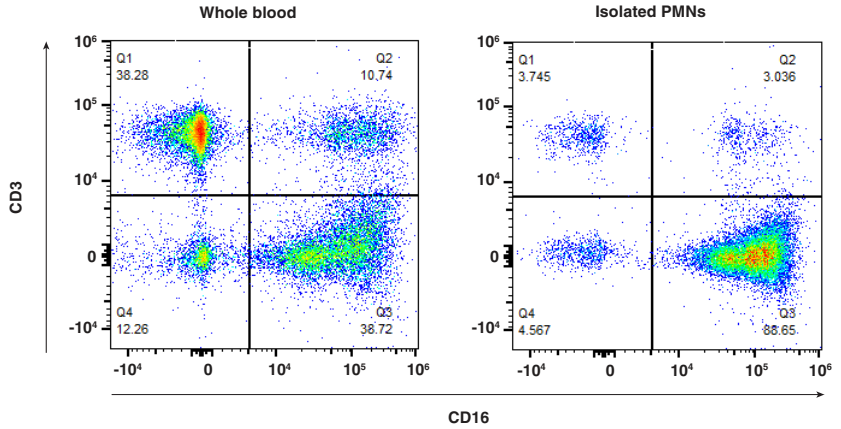

Figure 2. Enriched neutrophils obtained from Neutrophil Isolation Kit. Human whole blood was subjected to RBC lysis alone (left plot) or Neutrophil isolation (right plot). Resulting cells were stained for surface markers CD16 (neutrophils) and CD3 (T cells) and evaluated by flow cytometry. Neutrophils were enriched from less than 40% of the nucleated cell population to >88% of the population.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Poor viability of PMN	A. RBC lysis buffer incubation is too long B. Whole blood not fresh	A. Limit initial RBC lysis time B. Use blood within 3-4 hours of collection

NOTES

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.