EZPREP Peripheral Blood Mononuclear Cell Isolation Kit

Cat# EZPREP-10

INSTRUCTION MANUAL ZBM0117.01

STORAGE CONDITIONS

- **Reagents & Buffers:** Store at +4 to 30°C.

Read the label for each individual reagent for proper long-term storage conditions.

For *in vitro* Use Only

For Research Use Only. Not For Use in Diagnostic Procedures. Not for Human Therapeutic Use.

LIMITED PRODUCT WARRANTY

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Contact ZenBio, Inc. within no more than 24 hours after receipt of products for all claims regarding shipment damage, incorrect ordering or other delivery issues. Delivery claims received after 7 days of receipt of products are not subject to replacement or refund.

ORDERING INFORMATION AND TECHNICAL SERVICES

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- **PO Box 13888**
- **Research Triangle Park, NC 27709, USA**
- **Telephone** (919) 547-0692
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- **Toll Free** **1-866-ADIPOSE (866)-234-7673**
- **Electronic mail (e-mail)** **information@zen-bio.com**
- **World Wide Web** **http://www.zen-bio.com**

INTRODUCTION

The EZPREP Peripheral Blood Mononuclear Cell (PBMC) Isolation Kit is designed to isolate highly purified peripheral blood mononuclear cells (PBMCs) from fresh whole blood products by using density gradient centrifugation. Red blood cells (RBCs), platelets and unwanted cells are targeted for removal by the density gradient, lysis buffer, and a slow speed spin protocol. PBMCs are immediately available for downstream applications such as flow cytometry, DNA/RNA isolation or culture of subpopulations of cells. This kit is suitable for isolation of PBMC from a maximum of 100ml human whole blood.

ITEMS INCLUDED IN THE KIT

ITEM	STORAGE NOTES	UNIT	QUANTITY
EZPrep Tubes	Store at 4 to 30°C	EACH	10
Blood Diluent	Store at 4 to 30°C	120 ML BOTTLE	1
Lymphocyte Separation Medium	Store at 4 to 30°C	120 ML BOTTLE	1
Resuspension Buffer	Store at 4 to 30°C	250 ML BOTTLE	1
RBC Lysis Buffer	Store at 4 to 30°C	120 ML BOTTLE	1

NOTE: The solutions provided in this kit do not contain any animal or recombinant ingredients.

Other equipment/reagents required but not provided with the kit:

- **Anticoagulated human whole blood that is less than 24 hours old after collection.**
- Serological pipettes
- 50 mL STERILE conical centrifuge tubes
- Centrifuge Tube rack, *Note: Our racks are designed to be easily separated for use outside of the kit. Additionally, standard Styrofoam 50ml conical tube racks will also work well.*

PRECAUTIONS

This product is for research use only. It is not intended for human, therapeutic or diagnostic uses. Proper precautions and biological containment should be taken when handling blood products of human origin, due to their potential biohazardous nature. We recommend using the US Centers for Disease Control (CDC) Universal Precautions for prevention of blood-borne pathogens as a minimum guideline for standards of practice at Biosafety Level 1 or higher. Proper precautions and biological containment should be taken when handling blood of human origin, due to their potential biohazardous nature. All human based products should be handled at a BSL-1 (Biosafety Level 1) or higher. All work performed using this kit should be maintained in a sterile environment such as a Class 2 Biosafety Cabinet.

SAMPLE PREPARATION FROM HUMAN WHOLE BLOOD

1. **Bring all reagents and whole blood to room temperature prior to use.**
2. In a sterile environment, transfer 10 mL of anti-coagulated whole blood from the collection tube to a sterile 50 mL centrifuge tube. Rinse the collection tube with 10 mL of Blood Diluent and add to the 50 mL conical centrifuge tube. Cap the tube and mix by inversion 5 times.
3. Place an EZPrep Tube in a rack and add 10 mL of Lymphocyte Separation Medium to the bottom of the tube.
4. Carefully layer the diluted blood on top of the separation medium layer. Avoid any intermixing of separation medium and diluted blood.
5. Centrifuge tube at 700 x g for 30 minutes at 20°C with brake to “OFF” position for deceleration.
6. After centrifugation, carefully remove tube as not to disrupt the interface. Remove the thin band of PBMCs at the interface using a pipette removing no more than 3 mL and transfer to a new 50 mL centrifuge tube.
7. In the 50 mL tube containing the PBMCs, add 10 mL with Resuspension Buffer and centrifuge at 400 x g for 10 minutes at 20°C.
8. After centrifugation, carefully aspirate the supernatant without disturbing the pellet.
9. Re-suspend in 10 mL RBC Lysis Buffer to ensure adequate lysing of red blood cells. Let stand on ice for 10 minutes.
10. After 10 minutes, centrifuge tube at 400 x g for 10 minutes at 20°C. Carefully aspirate the supernatant without disturbing the pellet and re-suspend in 10 mL of Resuspension Buffer.
11. For removal of platelets (top layer opaque), centrifuge at 200 x g for 10 minutes at 20°C, Carefully aspirate supernatant and re-suspend in 5 mL Resuspension Buffer.
12. The cells are now ready for further processing, applications, or cryopreservation.

FREQUENTLY ASKED QUESTIONS/TROUBLESHOOTING

1. **Why is there an increased red blood cell contamination of the lymphocytes?** The processing temperature is too low. The Lymphocyte Separation Medium density is greater at low temperatures and granulocytes and RBCs are prevented form entering the separation medium. Increase the processing temperature to 18-20°C.

2. **Why is the lymphocyte yield and viability low??**The processing temperature is too high. The Lymphocyte Separation Medium density is less at high temperatures and the lymphocytes may penetrate into the density layer. Cell viability may also be affected.

3. **Why is the lymphocyte yield low but my viability high?** Blood has not been diluted to 1:1 with Blood Diluent. High cell density results in large numbers of lymphocytes being trapped by red blood cell aggregates. Dilute the blood sample further.

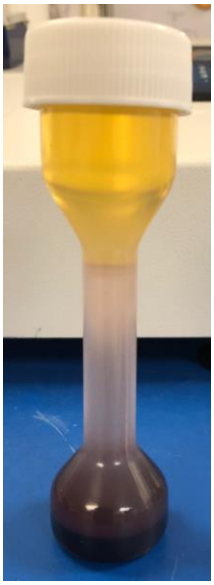
4. **Are the tubes reusable?** No. You can order the tubes separately and use your own gradient.

Catalog #	Description	Size
EZPREP-BULK	EZPREP Peripheral Blood Mononuclear Cell Isolation TUBES ONLY	Pack of 25

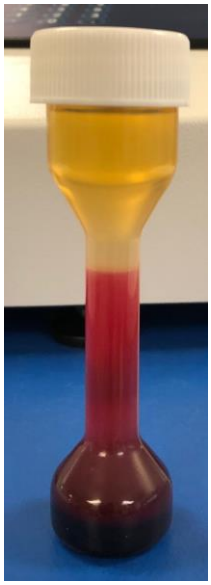
5. **Why did the RBCs not lyse in the RBC Lysis Buffer?** The RBC Lysis Buffer concentration is important. Insufficient aspiration of supernatant after centrifugation dilutes the buffer. Repeat lysis step to eliminate any remaining RBCs.

6. **Why did the gradient not work?** Improper layering of the diluted blood on the Lymphocyte Separation Medium causes intermixing and results in poor separation.

Good separation →



Poor separation



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