Normal Human Peripheral Blood Mononuclear Cells, Frozen

#79059

Description

Cryopreserved vial (100 x 10^6, 30 x 10^6, 10 x 10^6 or 2 x 10^6 cells) of freshly isolated primary human peripheral blood mononuclear cells (PBMCs) from a healthy donor, isolated from leukapheresis /apheresis samples using Ficoll gradient. After isolation, the PBMCs were stained to identify sub populations and evaluated for viability by flow cytometry. Cells were cryopreserved in serum-free Cryostor CS10 at a controlled rate.

Background

Human peripheral blood mononuclear cells (PBMCs) include all the cells present in the peripheral blood that have a round nucleus. It is thus a mix of lymphocytes, such as T, B and NK cells, and monocytes. Most cells present in PBMCs are lymphocytes, with T cells being the main cell type. The proportion of each cell type present can be determined by using cell specific marker combinations, for instance NK cells are characterized as $CD3^-CD56^+$ cells. The use of these markers also allows for isolation of cell populations of interest. PBMCs are a critical and widely used cell source in the immunology, infectious disease, vaccine, cancer therapy and transplant immunology fields.

Application

Studies involved PBMCs or isolated immune cells (such as T cells).

Source

140ml Leukopak blood

Donor Testing

Donors have been screened and determined negative for:

- Syphilis
- Red Blood Cell Antibody Screen
- Hepatitis B (anti-HBc EIA, HBsAg EIA)
- Hepatitis C (anti-HCV EIA)
- Human Immunodeficiency Virus (HIV-1/HIV-2 plus O)
- Human T-Lymphotropic Virus (HTLV-I/II)
- HIV-1/HCV/HBV
- West Nile Virus
- Trypanasoma cruzi

Note: *Testing cannot completely guarantee that any sample is completely virus-free. These cells should be treated as potentially infectious and appropriate biological safety level 2 (BSL2) precautions should be used.*

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 10	BPS Bioscience #79704

Storage Conditions

Cells will arrive upon dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -135°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics.
Thawed cells should be used immediately for downstream applications. As these are primary cells, we do not recommend maintaining these cells in culture for long periods of time.

Media Required for Cell Culture

Thaw Medium 10 (BPS Bioscience #79704):
RPMI 1640 medium supplemented with 10% FBS, 1mM Sodium pyruvate, 1% Non-essential amino acids, 1% Penicillin/streptomycin.

Cell Handling Protocol

Note: If cell pellets appear pinkish/light red it is due to the presence of a small fraction of Red Blood Cells that was isolated with the PBMC layer. If desired/necessary, these can be quickly and easily lysed while leaving the PBMC intact, by using RBC Lysis Buffer. RBC may not interfere with downstream PMBC culture experiments, but this will depend on the assay of interest.

Cell Thawing

1. Swirl the vial of frozen cells in a 37°C water bath until 90% of the vial is thawed.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Clean the outside of the vial with 70% ethanol.
3. As soon as the cells are thawed, quickly transfer the entire contents of the vial to a 15 ml tube.
4. Rince the vial with Thaw Medium 10 and add to the 15 ml tube.
5. Slowly add 10 ml of pre-warmed Thaw Medium 10.
6. Gently mix by inverting the tube.
7. Immediately spin down the cells at 200 *x g* for 10 minutes at Room Temperature (RT).
8. Carefully remove the supernatant with a pipette without disturbing the pellet.
9. Resuspend the pellet with 10 ml of pre-warmed Thaw Medium 10.
10. Gently mix by inverting the tube.
11. Spin down the cells at 200 *x g* for 10 minutes at RT.
12. Carefully remove the supernatant with a pipette without disturbing the pellet.

    Note: Up to 20% cell loss can be expected during washing steps.

13. Cells are now ready for downstream applications.

Characterization Data
Donor Demographics and Lot Specific Data

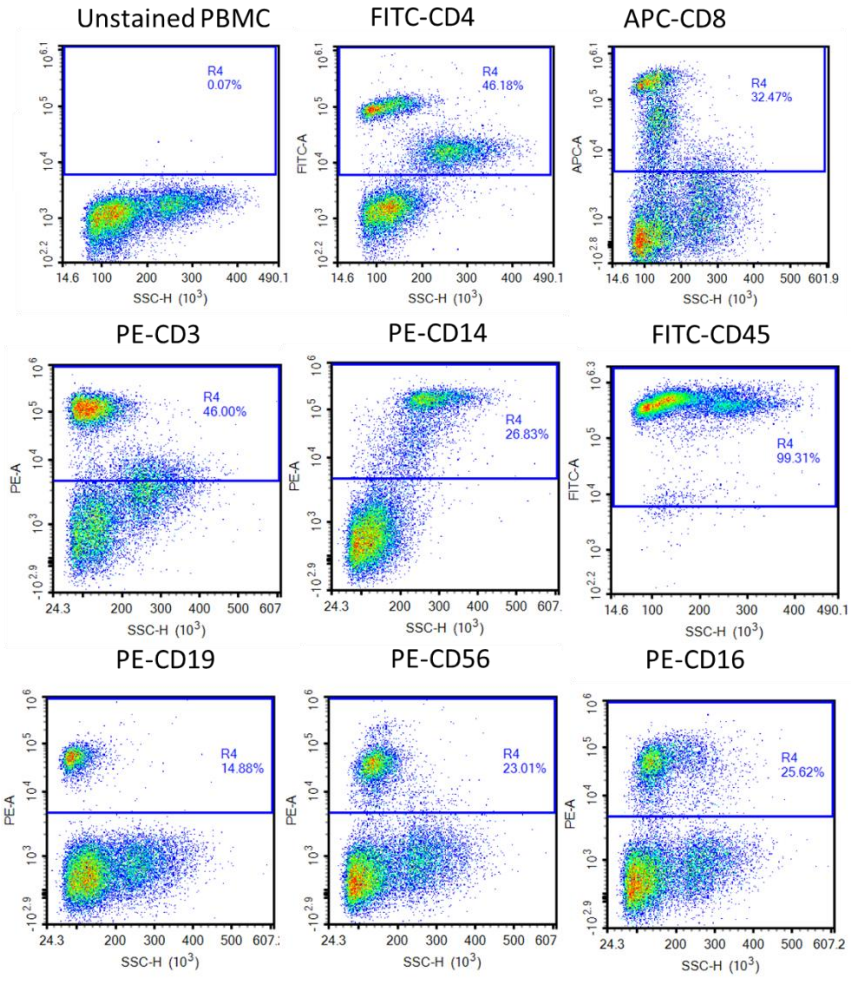
Donor Gender	Age	Ethnicity	Blood Type	Cryopreservation Date
Male	25	African American	O	Jan5, 2024

Surface Marker Summary

7-AAD Viability Dye	SYTOX$^-$ CD45$^+$	CD14$^+$ Monocytes	CD19$^+$ B cells	CD3$^+$ T Cells	CD4$^+$ Th Cells	CD8$^+$ Tc Cells	CD56$^+$ NK Cells
97.89%(Live)	99.31%	26.83%	14.88%	46.00%	46.18%	32.47%	23.01%

Flow Cytometry Analysis

- Results from frozen cells that were thawed and washed according to the handling directions.
- Cell count, viability (trypan blue exclusion and FACS with impermeable DNA binding dye), and surface markers (CD45$^+$, CD14$^+$, CD19$^+$, CD3$^+$, CD4$^+$, CD8$^+$, CD56$^+$).



Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
PBMC Cytotoxicity Bioassay (CFSE, 7-ADD)	82173	1 kit
NCAM1/CD56 Positive Cell Isolation Kit	78808	1 x 10^8 cells/1 x 10^9 cells
Anti-CD3 Agonist Antibody	71274	50 µg/100 µg
Anti-CD28 Agonist Antibody	100182	50 µg/100 µg
Anti-NCAM1 Antibody, PE-Labeled	101673	25 µg/100 µg
Anti-CD3 Antibody, FITC-Labeled	102008	25 µg/100 µg

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