Data Sheet

Normal Human Peripheral Blood Mononuclear Cells, Frozen
Catalog # 79059

Description
Cryopreserved vial (30 x 10^6 cells) of freshly isolated primary human peripheral blood mononuclear cells (PBMCs) from a healthy donor, isolated from whole blood or leukapheresis 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 FBS with 10% DMSO at a controlled rate.

Source
450mL whole blood

Stability and Storage
Store cells at -135˚C or colder. 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.

Characterization Criteria
Cell count, viability (trypan blue exclusion and FACS with impermeable DNA binding dye), and surface markers (CD45+, CD14+, CD19+, CD3+, CD4+, CD8+, CD56+)

Medium
Immuno Culture Medium (BPS Bioscience #79704): RPMI 1640 medium (Thermo Fisher, #A1049101) supplemented with 10% FBS (Thermo Fisher, #26140079), 1mM Sodium pyruvate (Corning, #25-000-CL), 1% Non-essential amino acids (Corning, #25-025-CL), 1% Penicillin/streptomycin (Thermo Fisher, #15140122)

Materials or Instruments Required but not Supplied:
Red Blood Cell Lysis buffer (if desired): If cell pellets appear pinkish/light red it is due to a small fraction of Red Blood Cells that did not isolate from 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.
Handling Directions

Prepare a 50 ml conical tube with 10 ml of pre-warmed medium (10% FBS in IMDM or LGM). Quickly thaw cells in a 37°C water bath with constant and slow agitation. It is important to work quickly in the following steps to ensure high cell viability and recovery. Clean the outside of the vial with 70% ethanol and immediately transfer the entire content of the tube into the medium. Gently swirl the tube and centrifuge the cell suspension at 300 x g for 10 minutes at room temperature. Carefully remove the supernatant with a pipette without disturbing the pellet. Gently resuspend the cell pellet in 15-20 mL of warm medium. Centrifuge the cell suspension at 300 x g for 10 minutes at room temperature. Carefully remove the supernatant with a pipette without disturbing the pellet. Gently resuspend the cell pellet in warm medium and mix by gently flicking the tube. **NOTE:** Up to 30% of cell loss can be expected during washing steps. Cells are now ready for use in downstream applications.

Donor Screening

Donors have been screened and determined negative for:

- 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
- Trypanosoma 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 precautions should be used.*

Donor Demographics and Lot Specific Information

<table>
<thead>
<tr>
<th>Donor Gender</th>
<th>Age</th>
<th>Ethnicity</th>
<th>Blood Type</th>
<th>Cryopreservation Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>58</td>
<td>Caucasian</td>
<td>O-POS</td>
<td>May 17, 2019</td>
</tr>
</tbody>
</table>

Surface Marker Summary

<table>
<thead>
<tr>
<th>7-AAD Viability Dye</th>
<th>CD14+ Monocytes</th>
<th>CD19+ B cells</th>
<th>CD3+ T Cells</th>
<th>CD4+ T Cells</th>
<th>CD8+ T Cells</th>
<th>CD45+ Cells</th>
<th>CD56+ NK Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>96.9% (live)</td>
<td>3.2%</td>
<td>3.8%</td>
<td>23.2%</td>
<td>18.1%</td>
<td>18.3%</td>
<td>33.6%</td>
<td>0.8%</td>
</tr>
</tbody>
</table>

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Flow Cytometry Analysis

Results from frozen cells that were thawed and washed according to the handling directions