

## Primary Cells

### G-CSF and Plerixafor Mobilized Human Peripheral Blood Mononuclear Cells, Frozen

|           |         |                             |
|-----------|---------|-----------------------------|
| Catalog # | 70072.4 | 5 x 10 <sup>6</sup> cells   |
|           | 70072.2 | 1.5 x 10 <sup>7</sup> cells |
|           | 70072.3 | 2.5 x 10 <sup>7</sup> cells |
|           | 70072.1 | 5 x 10 <sup>7</sup> cells   |
|           | 70072   | 1 x 10 <sup>8</sup> cells   |



Scientists Helping Scientists™ | [WWW.STEMCELL.COM](http://WWW.STEMCELL.COM)

TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713

[INFO@STEMCELL.COM](mailto:INFO@STEMCELL.COM) • [TECHSUPPORT@STEMCELL.COM](mailto:TECHSUPPORT@STEMCELL.COM)

FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

## Product Description

Primary human mononuclear cells (MNCs) were isolated from mobilized peripheral blood (PB) leukapheresis samples using density gradient separation or red blood cell lysis. Donors were mobilized with:

- Catalog #70049: a maximum of 10 µg/kg/day of granulocyte colony-stimulating factor (G-CSF) for 3 - 5 days prior to collection
- Catalog #70074: a maximum of 0.24 mg/kg of plerixafor for 1 day prior to collection
- Catalog #70072: a maximum of 10 µg/kg/day of G-CSF for 3 - 5 days prior to collection and a maximum of 0.24 mg/kg of plerixafor for 1 day prior to collection

Cells were obtained using Institutional Review Board (IRB)-approved consent forms and protocols.

|                            |  |
|----------------------------|--|
| Donor Status:              | Mobilized with G-CSF and plerixafor  |
| Characterization Criteria: | Cell count, viability, donor virus testing, age, sex, ethnicity, weight, height, smoking status, other information |
| Format:                    | MNCs are frozen in CryoStor® CS10  |
| Anticoagulant:             | Acid citrate dextrose solution A (ACDA)  |

For donor details, refer to the lot-specific Certificate of Analysis.

## Stability and Storage

Product stable at -135°C or colder for 12 months from date of receipt. Short-term storage of cells (< 1 month) at -80°C is acceptable, but should be minimized to ensure maximum stability. Thawed samples must be used immediately. As these are primary cells, they have a finite life span in culture.

## Precautions

Donor Screening: Donors are screened for HIV-1, HIV-2, hepatitis B, and hepatitis C.

Cryopreserved products are shipped with negative test results from donor screening that is performed within 90 days of collection.

Donors have been tested and found to be negative for HIV-1, HIV-2, hepatitis B, and hepatitis C prior to donation. As testing cannot completely guarantee that the donor was virus-free, THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS and only used following appropriate handling precautions such as those described in biological safety level 2.

Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross-contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cells from one container to another. When handling this product, do not use sharps such as needles and syringes.

STEMCELL cannot guarantee the biological function or any other properties associated with performance of cells in a researcher's individual assay or culture systems. STEMCELL assures the cells will meet the specifications only when assessed immediately after thawing (before washing) by our test methods.

FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS.  
NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

## Handling / Directions for Use

**IMPORTANT:** To confirm the number of cells provided, a viable cell count must be done immediately after thawing (before washing). Work quickly once the cells have been thawed to ensure high viability and recovery. Use sterile techniques when processing thawed cells.

1. Warm medium in a 37°C water bath. See Accessory Products (below) for recommended media.
2. Wipe the outside of the vial of cells with 70% ethanol or isopropanol.
3. In a biosafety hood, twist the cap a quarter-turn to relieve internal pressure and then retighten.
4. Quickly thaw cells in a 37°C water bath by gently shaking the vial. Remove the vial when a small frozen cell pellet remains. Do not vortex cells.  
NOTE: It is important to work quickly in the following steps to ensure high cell viability and recovery.
5. Wipe the outside of the vial with 70% ethanol or isopropanol.
6. Measure the total volume of the cell suspension using a 2 mL serological pipette. This value is used in step 12 to calculate the number of cells provided.
7. Remove a 20 µL aliquot of cells for counting. If using Trypan Blue to assess viability, for  $\geq 1 \times 10^6$  cells we suggest adding a minimum of 20 µL of medium and recording the volume of medium added. For  $< 1 \times 10^6$  cells, dilute directly in 20 µL Trypan Blue. Set diluted aliquot aside until step 12. See Tips section for more details on performing cell counts with a hemocytometer.
8. Transfer the remaining cell suspension to a 50 mL conical tube.
9. Rinse the vial with 1 mL of medium and add it dropwise to the cells, while gently swirling the 50 mL tube.
10. Wash by adding 15 - 20 mL of medium dropwise, while gently swirling the tube.
11. Centrifuge the cell suspension at 300 x g for 10 minutes at room temperature (15 - 25°C).
12. If using Trypan Blue, perform a cell count on the diluted aliquot from step 7.
13. Carefully remove the supernatant (from step 11) with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.
14. If cells are starting to clump, add 100 µg DNase I Solution per mL of cell suspension and incubate at room temperature (15 - 25°C) for 15 minutes.  
NOTE: Do not add DNase I Solution if the cells will be used for DNA or RNA extraction.
15. Gently add 15 - 20 mL of medium to the tube.
16. Centrifuge the cell suspension at 300 x g for 10 minutes at room temperature (15 - 25°C).
17. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.  
NOTE: Cell loss of up to 30% can be expected during the wash steps.
18. Cells are now ready for use in downstream applications.

## Tips

Manual cell counting protocol: <http://www.humanimmunologyportal.com/protocols/performing-cell-counts-with-a-hemacytometer/>

Converting g to rpm: <http://www.humanimmunologyportal.com/hiptools/apps/>

## Accessory Products

| PRODUCT NAME   | CATALOG # |
|--|-----------|
| Iscove's Modified Dulbecco's Medium (add 10% fetal bovine serum) | 36150     |
| DMEM with 4500 mg/L D-Glucose (add 10% fetal bovine serum)       | 36250     |
| RPMI 1640 Medium (add 10% fetal bovine serum)                    | 36750     |
| Trypan Blue  | 07050     |
| DNase I Solution (1 mg/mL)                                       | 07900     |
| Falcon® Serological Pipettes, 2 mL                               | 38002     |
| Falcon® Conical Tubes, 50 mL                                     | 38010     |

STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485. PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED.

Copyright © 2018 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, and Scientists Helping Scientists are trademarks of STEMCELL Technologies Canada Inc. CryoStor is a registered trademark of BioLife Solutions. All other trademarks are the property of their respective holders. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.