Primary Cells

Human Peripheral Blood Leukopak, Frozen

Catalog #200-0130	Full Size
200-0131	Half Size
200-0132	Quarter Size
200-0470	Tenth Size

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

Leukapheresis is performed on normal donors using Institutional Review Board (IRB)- or Research Ethics Committee (REC)-approved consent forms and protocols. Approximately two to three blood volumes are processed using the Spectra Optia® Apheresis System to produce a full-sized Leukopak. The collected product is then cryopreserved in a controlled-rate freezer.

Donor Status:	Normal
Characterization Criteria:	Cell count, viability, donor virus testing, age, sex, ethnicity, weight, height, smoking status, other information
Format:	Product is drawn into a sample collection bag containing anticoagulant and is 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 is shipped on dry ice with liquid nitrogen shipping available upon request. For best results, use product immediately upon receipt. Otherwise, store at -135°C or colder.

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 and 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.

Directions for Use

IMPORTANT: To confirm the number of cells provided, a viable cell count must be done immediately after thawing. Work quickly once the Leukopak has been thawed to ensure high viability and recovery. Use sterile technique when processing thawed Leukopak.

WARNING: Handle frozen Leukopaks with care and inspect thoroughly upon receipt. Ensure the cryobag, tubing, and ports are intact and properly sealed prior to thawing. Freezing causes the plastic to become rigid, and while rare, can lead to breakage if improperly handled. Use extra care and avoid impacts when handling, transporting, and thawing the frozen product.

The following instructions are for thawing Leukopaks. Instead of using a water bath (steps 3 - 4), frozen Leukopaks can be thawed using ThawSTAR® CB Automated Thawing System (Catalog #100-1151). For complete instructions, refer to the Product Information Sheet (Document #10000018736), available at www.stemcell.com, or contact us to request a copy.

1. Warm sufficient volume of HBSS Modified (Without Ca++ and Mg++) + 10% fetal bovine serum (FBS) in a 37°C water bath. Wipe with 70% ethanol and transfer to the biosafety cabinet.
2. Add DNase I to prepare thawing medium (HBSS Modified [Without Ca++ and Mg++] + 10% FBS + 0.1 mg/mL DNase I [final concentration]).

   NOTE: Do not add DNase I if the cells will be used for DNA or RNA extraction.

   NOTE: Thawing medium must be prepared fresh before each use.
3. Remove frozen Leukopak from liquid nitrogen storage and immediately place in a 37°C water bath. Submerge the Leukopak, but keep the ports elevated above the waterline, and do not agitate the bag while it is thawing.
4. When the Leukopak is mostly thawed (with a small amount of ice remaining), remove from the water bath and wipe the outside of the bag with 70% ethanol.
5. In a biosafety cabinet, slowly transfer the cell suspension into a sterile bottle that can contain 5 - 10 times the volume of the Leukopak, using the port on the bottom of the cryobag.
6. While gently swirling the cell suspension, add an equal volume of thawing medium dropwise to the bottle.
7. Optional: To improve recovery, rinse bag by adding one-half volume of thawing medium (relative to original Leukopak volume) to the Leukopak bag. Mix thoroughly and transfer to the cell suspension in the sterile bottle.
8. Slowly add an additional two volumes (relative to original Leukopak volume) of thawing medium to the cell suspension.
9. Thoroughly mix the sample (do not vortex).
10. Measure the volume precisely. Remove a small aliquot of cell suspension for counting and viability assessment. See Notes and Tips section for performing cell counts with a hemocytometer.
11. Transfer the cell suspension to 50 mL conical tubes and centrifuge at 300 x *g* for 10 minutes at room temperature (15 - 25°C).
12. 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.
13. Optional: If cells are starting to clump, add 0.1 mg DNase I per mL of cell suspension and incubate for 15 minutes at room temperature.
14. Gently add at least 15 - 20 mL of thawing medium to each tube.
15. Centrifuge the cell suspension at 300 x *g* for 10 minutes at room temperature.
16. Carefully remove the supernatant 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 and adding desired medium.

    NOTE: Cell losses are expected during the wash steps.
17. Cells are now ready for use in downstream applications.

    NOTE: Platelet and red blood cell (RBC) levels will vary between samples, and further processing may be required prior to use indownstream applications (i.e. slow spins to remove platelets, RBC lysis, or density gradient separation). See Notes and Tips section for an optional RBC Lysis and Platelet Removal protocol (after diluting and centrifuging the sample).

Notes and Tips

For a protocol on performing total nucleated cell counts using a hemocytometer, refer to https://www.stemcell.com/how-to-count-cells-with-a-hemocytometer.

For a protocol on RBC Lysis and Platelet Removal, refer to "Processing a Leukopak for Downstream Cell Isolation" (Part II: Prepare Leukopak Contents, Option 2), available at https://www.stemcell.com/leukopak-processing-protocol.html

Accessory Products

PRODUCT NAME	CATALOG #
DNase I	07469
Falcon® Conical Tubes, 50 mL	38010
Falcon® Serological Pipettes, 2 mL	38002
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
HBSS, Modified (Without Ca++ and Mg++)	37250
ThawSTAR® CB Automated Thawing System	100-1151
Trypan Blue	07050