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Human CD34+ Cells

Technical information and instructions for use

Safety Statements

These products are not for use in GMP manufacturing, nor human or animal *in vivo* use, including use as a diluent or as an excipient, or for diagnostic use.

These products are for *research use only.*

WARNING: LONZA PRIMARY CELLS CONTAIN HUMAN SOURCE MATERIAL; TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-I, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV-1, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure to potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th edition. If you require further information, please contact your site safety officer or Scientific Support.

I. Introduction

Lonza offers highly purified CD34+ progenitor cells isolated from cord blood, bone marrow, and mobilized leukopaks. Cord blood is obtained directly from the umbilical vein either before or after delivery of the placenta. Bone marrow is obtained from normal donors by bilateral aspirates of the posterior iliac crest. Mobilized leukopaks are collected from adult blood donors via apheresis. Donors are screened for general health, normal blood counts, and infectious diseases as part of the collection process.

CD34+ selected cells are a highly purified population of progenitor cells and allow for greater cell expansion and differentiation along multiple pathways. CD34+ and CD133+ progenitors can differentiate into a number of precursor and specialized cell types.

Hematopoietic progenitors express high levels of the cell surface glycoprotein CD34. As these cells mature and differentiate, the levels of CD34 decrease. CD34+ cells can initiate long-term hematopoiesis and are critical in the drug-discovery process.

Lonza offers CD34+ progenitors isolated from mononuclear cells using positive immunomagnetic selection. CD34+ progenitors are available cryo-preserved in quantities starting from ≥100,000 cells/order. Purity (as determined by flow cytometry) for bone marrow, cord blood, and mobilized leukopak-derived CD34+ progenitors is ≥90%.

For answers to frequently asked questions and citations regarding these products, please visit our Knowledge Center: **https://knowledge.lonza.com**

II. Suggested Reagents

(Components sold separately)

Medium and Supplements:

1. X-VIVO® 20 Hematopoietic Growth Medium – 1 L (Lonza Catalog No. 04–448Q) with ITES (ThermoFisher GIBCO™ 51500056 or similar)

   OR

2. IMDM (Iscove's Modified Dulbecco's Medium) – 500 mL (Lonza Catalog No. 12–722F) with

HyClone™ Fetal Bovine Serum U.S. Origin (Cytiva Catalog No. SH30071.01) or similar

Cytokines for Proliferation:

1. Recombinant Human TPO (ThermoFisher Catalog No. PHC9511 or similar)
2. Recombinant Human SCF (ThermoFisher Catalog No. PHC2111 or similar)
3. Recombinant Human FLT-3 Ligand (Sigma-Aldrich Catalog No. SRP3044 or similar)

III. General Cell Information and Quality Control

Cat. No.	Description	Cells/Vial
2M-101	Cryopreserved Bone Marrow CD34+ Cells	≥100,000 viable cells
2M-101A	Cryopreserved Bone Marrow CD34+ Cells	≥300,000 viable cells
2M-101B	Cryopreserved Bone Marrow CD34+ Cells	≥500,000 viable cells
2M-101C	Cryopreserved Bone Marrow CD34+ Cells	≥1 million viable cells
2M-101D	Cryopreserved Bone Marrow CD34+ Cells	≥2 million viable cells
2C-101A	Cryopreserved Cord Blood CD34+ Cells	≥500,000 viable cells
2C-101B	Cryopreserved Cord Blood CD34+ Cells	≥100,000 viable cells
2C-101	Cryopreserved Cord Blood CD34+ Cells	≥1 million viable cells
2C-101X	Cryopreserved Cord Blood CD34+ Cells	≥1 million viable cells, ≥2 vials
2C-101H	Cryopreserved Cord Blood CD34+ Cells w/HLA characterization	≥1 million viable cells
2C-101HX	Cryopreserved Cord Blood CD34+ Cells w/HLA characterization	≥1 million viable cells, ≥2 vials
2Y-101C	Cryopreserved Mobilized Peripheral Blood CD34+ Cells	≥1 million viable cells
2Y-101D	Cryopreserved Mobilized Peripheral Blood CD34+ Cells	≥5 million viable cells
2Y-101E	Cryopreserved Mobilized Peripheral Blood CD34+ Cells	≥10 million viable cells
2Y-101F	Cryopreserved Mobilized Peripheral Blood CD34+ Cells	≥25 million viable cells
Characterization: Total cell population is **≥90% CD34+** as determined by flow cytometry. **Recommended Culture Medium:** X-VIVO® 20 Medium (w/ITES) or IMDM (w/15% FBS).		

All cells are performance assayed and test negative for HIV-1, Hepatitis-B, and Hepatitis-C. Certificates of Analysis (COA) for each product are found on the Lonza Biosciences **website**. Please see Section IX (Product warranty and safety, Page 4) for more information on product warranty and performance guarantees.

IV. Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells: Remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact Customer Service.
3. X-VIVO® 20 Medium instructions: Store medium at 2° to 8°C in the dark. **Do not freeze**.

V. Preparation of culture media

NOTE: Expansion of cells may lead to decreased CD34+ expression

1. Decontaminate external surfaces of all vials and the medium bottle with ethanol or isopropanol.
2. To formulate X-VIVO® 20 Medium (w/ ITES) or IMDM (w/15% FBS) for culturing CD34+ cells, add SCF to a final concentration of 25 ng/mL, TPO to a final concentration of 50 ng/mL, and FLT-3 to a final concentration of 50 ng/mL.

3. After cytokines are added to basal medium, store at 2° to 8°C and use within 1 week. **Do not freeze medium**.

NOTE: If there is concern that sterility was compromised during the supplementation process, the entire newly prepared culture medium may be re-filtered with a 0.2 µM filter to assure sterility. Routine re-filtration is not recommended.

VI. Thawing of Cells

1. Warm 10 mL of X-VIVO® 20 Medium (w/ ITES) or IMDM (w/ **10% FBS**) to room temperature in a 15 mL conical tube.
2. Wipe the outside of the cryovial 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 the vial of frozen cells in a 37°C water bath for no more than 2 minutes until a sliver of ice remains. Wipe the outside of the vial with 70% ethanol.

NOTE: In the following steps, it is important to work quickly to ensure high cell viability and recovery. Do not thaw more than 4 amps at the same time.

5. Transfer contents of the vial to the 15 ml conical tube of medium from step 1, taking care to minimize the pipetting of cells in cryopreservation medium as they are fragile.
    a. Perform this step rapidly! Do not keep cells in original cryopreservation medium for an extended period of time. This can reduce post-thaw recovery and viability.
6. Rinse the vial with approximately 1 mL of medium from the 15 mL conical tube and add it back to the same 15 mL tube.
7. Centrifuge at 300x*g* for 10 minutes at 2° to 8°C.

NOTE: Cell loss of up to 30% can be expected from the wash procedure.

8. Carefully aspirate supernatant, removing as much residual medium as you can without disturbing the cell pellet.
9. Add 1 mL of cell culture medium (X-VIVO® 20 with ITES or IMDM w/**15% FBS**) to the 15 mL tube. Gently flick the tube to resuspend the pellet.
10. Perform a cell count.
    a. Lonza strongly recommends using a hemocytometer and 0.4% Trypan Blue solution in a 1:1 ratio; e.g., 20 µL cell suspension and 20 µL Trypan Blue solution. Combine both in a separate microcentrifuge tube and mix with the pipettor. Pipet 10 µL of mixture into each chamber of the hemocytometer and perform a cell count.
        i. Adjust the volume of media used to resuspend cells and/or the volume of Trypan Blue solution added so that between 100 and 500 cells are present in the hemocytometer grid.

NOTE: Automated cell counters may be used instead of a hemocytometer; however, it is critical that the cell counter is calibrated against both live and dead cell controls prior to use. Lonza strongly recommends establishing an SOP outlining the correct start up, calibration, reagent concentrations, and dilutions for your individual automated cell counter that has been validated against hemocytometer counts to ensure accuracy and consistency. Failure to do so can result in inaccurate measurements of cell count and/or viability.

NOTE: Lonza strongly recommends verifying cell counts that fall below advertised specifications on a hemocytometer if those results were determined using an automated cell counter.

11. Adjust final volume of cell suspension as appropriate for downstream applications.

VII. Initiation of Culture Process and Maintenance

NOTE: This procedure is a recommendation only. Lonza CD34+ cells are not quality control tested for proliferation, and proliferation is not guaranteed under the cell warranty. Expansion of cells may lead to decreased CD34+ expression.

1. Seed cells in the complete X-VIVO® 20 Medium (w/ ITES) or IMDM (w/ 15% FBS) at a density of 1 x 10^5 cells/mL.

NOTE: For expansion, the culture medium should contain additional cytokines listed in Section VI.

2. Replace the media every 3–4 days.
3. Maintain cultures between 0.1–1.5 x 10^6 cells/mL.
4. Passage cells 2 times per week.

a. Centrifuge cells at 300x*g* at room temperature for 10 minutes.

b. Carefully remove supernatant without disturbing cell pellet.

c. Resuspend cells in fresh media and perform a cell count (see Section VII, steps 8–11).

d. Reseed cells at density of 1 x 10^5 cells/mL.

VIII. Ordering information

Visit **https://bioscience.lonza.com** to place an order or for further information on the following products.

Normal human CD34+ cells:

Cat. No.	Product	Description
2M-101	Cryopreserved Bone Marrow CD34+ Cells	≥ 100,000 viable cells
2M-101A	Cryopreserved Bone Marrow CD34+ Cells	≥ 300,000 viable cells
2M-101B	Cryopreserved Bone Marrow CD34+ Cells	≥ 500,000 viable cells
2M-101C	Cryopreserved Bone Marrow CD34+ Cells	≥ 1 million viable cells
2M-101D	Cryopreserved Bone Marrow CD34+ Cells	≥ 2 million viable cells
2C-101A	Cryopreserved Cord Blood CD34+ Cells	≥ 500,000 viable cells
2C-101B	Cryopreserved Cord Blood CD34+ Cells	≥ 100,000 viable cells
2C-101	Cryopreserved Cord Blood CD34+ Cells	≥ 1 million viable cells
2C-101X	Cryopreserved Cord Blood CD34+ Cells	≥ 2 million viable cells per batch
2C-101H	Cryopreserved Cord Blood CD34+ Cells w/HLA characterization	≥ 1 million viable cells
2C-101HX	Cryopreserved Cord Blood CD34+ Cells w/HLA characterization	≥ 2 million viable cells per batch
2Y-101C	Cryopreserved Mobilized Peripheral Blood CD34+ Cells	≥ 1 million viable cells
2Y-101D	Cryopreserved Mobilized Peripheral Blood CD34+ Cells	≥ 5 million viable cells
2Y-101E	Cryopreserved Mobilized Peripheral Blood CD34+ Cells	≥ 10 million viable cells
2Y-101F	Cryopreserved Mobilized Peripheral Blood CD34+ Cells	≥ 25 million viable cells

CD34+ Cell Culture Media/Supplements:

Cat. No.	Product	Description
04–448Q	X-VIVO® 20 Medium	X-VIVO® 20 serum-free hematopoietic cell medium
12–722F	IMDM	500 mL Iscove's modified Dulbecco's medium
A variety of growth factors may be used, including G-CSF, GM-SCF and SCF. Multiple growth factors are required for optimum growth.		

IX. Product Warranty

Cultures have a finite lifespan *in vitro.*

Lonza guarantees the performance of our primary cells up to two years from purchase only if Lonza's recommended media and reagents are used exclusively and the recommended storage and use protocols are followed. Any modifications made to the recommended cell systems including the use of alternative media, reagents, or protocols will void cell and media performance guarantees. If you need assistance in selecting the appropriate media, reagents, or protocol, please contact Lonza Scientific Support.

When placing an order or contacting Lonza Scientific Support, please refer to the product numbers and descriptions listed above. For a complete list of all primary cell and media products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, for additional information, or to speak with Lonza Scientific Support, you can contact Lonza by web, e-mail, telephone, or mail (See page 1 for details).

ITES (GIBCO™ ITS-X; Thermo Fisher 51500056) is a product of GIBCO™.

HyClone™ FBS (Cytiva SH30071.01) is a product of Cytiva.

Recombinant Human TPO (Thermo Fisher PHC9511) is a product of Thermo Fisher Scientific.

Recombinant Human SCF (Thermo Fisher PHC 2111) is a product of Thermo Fisher Scientific.

Recombinant Human Flt-3 Ligand (Millipore Sigma SRP3044) is a product of Millipore Sigma.