

## Human CD34+ Cells

### Technical information and instructions

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#### I. Introduction

Bone marrow is obtained from normal donors by bilateral aspirates of the posterior iliac crest. Donors are screened for general health, normal blood counts and infectious diseases prior to collection.

CD34<sup>+</sup> selected cells are a highly purified population of progenitor cells and allow for greater cell expansion and differentiation down multiple pathways. CD34<sup>+</sup> and CD133<sup>+</sup> progenitors can differentiate into a number of precursors and differentiated 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<sup>+</sup> cells are capable of initiating long-term hematopoiesis both *in vitro* and *in vivo* and are therefore utilized in the therapeutic re-constitution of bone marrow.

Lonza offers CD34<sup>+</sup> progenitors isolated from mononuclear cells using positive immunomagnetic selection. CD34<sup>+</sup> progenitors are available cryopreserved in quantities starting from ≥100,000 cells/order from single or multiple donors. Purity is ≥95% for bone marrow derived cells and ≥90% for cord blood derived cells.

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)

- X-VIVO™ 20 Hematopoietic Growth Medium – 1 L (Lonza Catalog No. 04-448Q) with ITES (Lonza catalog # 17-839Z) or IMDM Iscove's Modified Dulbecco's Medium – 500 mL (Lonza Catalog No. 12-722F) with HyClone™ Fetal Bovine Serum U.S. Origin (GE Catalog No. SH30071.01 or similar)

##### Cytokines for Proliferation

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

Lonza guarantees the performance of these cells only if appropriate 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.

### III. General cell information

Cat. no.	Description	Recommended culture medium
1M-101C	Fresh Bone Marrow CD34+ Cells	X-VIVO™ 20 Medium (w/ ITES) or IMDM (w/15% FBS)
2M-101	Cryopreserved Bone Marrow CD34+ Cells	X-VIVO™ 20 Medium (w/ ITES) or IMDM (w/15% FBS)
2M-101A	Cryopreserved Bone Marrow CD34+ Cells	X-VIVO™ 20 Medium (w/ ITES) or IMDM (w/15% FBS)
2M-101B	Cryopreserved Bone Marrow CD34+ Cells	X-VIVO™ 20 Medium (w/ ITES) or IMDM (w/15% FBS)
2M-101C	Cryopreserved Bone Marrow CD34+ Cells	X-VIVO™ 20 Medium (w/ ITES) or IMDM (w/15% FBS)
2M-101D	Cryopreserved Bone Marrow CD34+ Cells	X-VIVO™ 20 Medium (w/ ITES) or IMDM (w/15% FBS)
2C-101A	Cryopreserved Cord Blood CD34+ Cells	X-VIVO™ 20 Medium (w/ ITES) or IMDM (w/15% FBS)
2C-101B	Cryopreserved Cord Blood CD34+ Cells	X-VIVO™ 20 Medium (w/ ITES) or IMDM (w/15% FBS)
2C-101	Cryopreserved Cord Blood CD34+ Cells	X-VIVO™ 20 Medium (w/ ITES) or IMDM (w/15% FBS)

### IV. Quality control

Cat. No.	Description	Cells/Vial	Characterization
1M-101C	Fresh Bone Marrow CD34+ Cells	≥1 million viable cells	CD34
2M-101	Cryopreserved Bone Marrow CD34+ Cells	≥100,000 viable cells	CD34
2M-101A	Cryopreserved Bone Marrow CD34+ Cells	≥300,000 viable cells	CD34
2M-101B	Cryopreserved Bone Marrow CD34+ Cells	≥500,000 viable cells	CD34
2M-101C	Cryopreserved Bone Marrow CD34+ Cells	≥1 million viable cells	CD34
2M-101D	Cryopreserved Bone Marrow CD34+ Cells	≥2 million viable cells	CD34
2C-101A	Cryopreserved Cord Blood CD34+ Cells	≥500,000 viable cells	CD34
2C-101B	Cryopreserved Cord Blood CD34+ Cells	≥100,000 viable cells	CD34
2C-101	Cryopreserved Cord Blood CD34+ Cells	≥1 million viable cells	CD34

All cells are performance assayed and test negative for HIV-1 and Hepatitis-B. Certificates of Analysis (COA) for each cell strain are shipped with each order. COAs for all other products are available upon request. Please see Section XI (Product Warranty, Page 4) for more information on Quality Control claims and guarantees.

### V. 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°-8°C in the dark. Do not freeze.

### VI. Preparation of culture media

**NOTE:** Expansion of cells may lead to a decrease of 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.

- After cytokines are added to basal medium, store at 2°-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.

## VII. Thawing of cells

- Warm 10 mL of X-VIVO™ 20 Medium (w/ ITES) or IMDM (w/ 10% Serum) in a 37°C water bath in a 15 mL conical tube.
- Wipe the outside of the cryovial with 70% ethanol or isopropanol.
- In a biosafety hood, twist the cap a quarter turn to relieve internal pressure, and then retighten.
- Quickly thaw the vial of frozen cells in a 37°C water bath for no more than 2 minutes. Wipe the outside of the vial with 70% ethanol.

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

- Remove 20 µL from the cryovial for cell counting.

**NOTE:** Counting cells taken directly from the vial is optional. Counting for Step 5 should be performed by another person while someone else is continuing with recovery of the cells.

- Aseptically transfer the contents of the cryovial to the conical tube with the pre-warmed media.
- Centrifuge the cell suspension at 300 X g at room temperature (15-25°C) for 10 minutes.

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

- Carefully remove the supernatant from Step 7 with a pipette, leaving a small amount of medium to ensure the pellet is not disturbed.
- Resuspend the cell pellet by gently flicking the tube.
- Add an appropriate volume of desired media and perform a cell count.
- Cells are now ready for use in downstream applications.

## VIII. 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 a decrease of CD34+ expression.

- Seed the cells in complete X-VIVO™ 20 Medium (w/ ITES) or IMDM (w/ 15% serum) at a density of  $1 \times 10^5$  cells/mL.
- For expansion, the culture medium should contain additional cytokines listed in Section VI.
- Replace the media every 3-4 days.
- Maintain cultures between  $0.1-1.5 \times 10^6$  cells/mL.
- Passage the cells 2 times a week.
- For passaging, centrifuge the cells at 300 X g at room temperature for 10 minutes and resuspend the cells in fresh media.

## IX. Ordering information

### Cryopreserved normal human dendritic cells

Cat. No.	Product	Description
1M-101C	Fresh Bone Marrow CD34+ Cells	≥1 million viable cells
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

## 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
17-839Z	ITES	Insulin, transferrin, selenium and ethanolamine, 5 mL

There are a variety of growth factors that may be used including G-CSF, GM-SCF and SCF. Multiple growth factors are required for optimum growth.

Mentioned products such as Recombinant Human TPO and Recombinant Human SCF are products from Thermo Fisher Scientific, Recombinant Human Flt-3 Ligand is a product of Sigma Aldrich, all other trademarks belong to Lonza or its affiliates or to their respective third party owners.

## X. Product warranty

Cultures have a finite lifespan *in vitro*.

Lonza guarantees the performance of our primary cells only if appropriate 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 for Scientific Support, please refer to the product numbers and descriptions listed above. For a complete listing 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 Scientific Support, you may contact Lonza by web, e-mail, telephone, or mail (See page 1 for details).

**THESE PRODUCTS ARE FOR RESEARCH USE ONLY.** Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* diagnostic procedures.

**WARNING: PRODUCTS 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-1 and hepatitis B virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, and hepatitis B 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 of potentially infectious products, as recommended in the CDC-NIH manual, [Biosafety in Microbiological and Biomedical Laboratories](#), 5<sup>th</sup> ed. If you require further information, please contact your site safety officer or Scientific Support.