Expansion of primitive Hematopoietic Progenitor Cells

Application Note

Background

Hematopoietic Stem/Progenitor Cells (HSCs/HPCs) represent a heterogeneous population of primitive blood progenitor cells that fall into several different classes [1,2]. Within the human body, they are mainly located in the bone marrow of adults, but are also found in various fetal tissues, e.g., umbilical cord blood, placenta and fetal liver.

HPCs are functionally defined by their self- renewal capacity and their multipotency allowing the replenishment of all types of blood cells. The myeloid branch of their descendants is represented by monocytes/macrophages, granulocytes (neutrophils, basophils, eosinophils), erythrocytes, megakaryocytes (platelet producer cells) and dendritic cells. The lymphoid branch comprises of T-lympho- cytes, B-lymphocytes and NK-cells [3]. Treatments using HPCs, e.g. bone marrow transplantation, have been used for over 40 years now and are well-established [4]. Indeed, HPSCs still hold great potential for further applications in regenerative medicine, e.g. "artificial blood", and are therefore intensively investigated by the scientific community.

It is known that *in vivo* HPCs are able to expand to very large numbers of daughter cells by virtue of their pronounced self-renewal abilities [5]. However, researchers still face difficulties while expanding HPSCs in traditional serum-containing media, as the cells tend to differentiate under these conditions. Our Hematopoietic Progenitor Cell (HSC/ HPC) HPC Expansion Medium XF (Cat. No. C-28021) provides a highly optimized, serum-free and xeno- free culture system allowing superior expansion performance of human HPCs, e.g. $CD34^+$ and $CD133^+$ cells. As a result, the expanded cell population is enriched with functional progenitor cells capable of colony forming cell (CFC)-formation in appropriate *in vitro* assays.

The medium is serum-free / xeno-free and does not contain any animal-derived components.

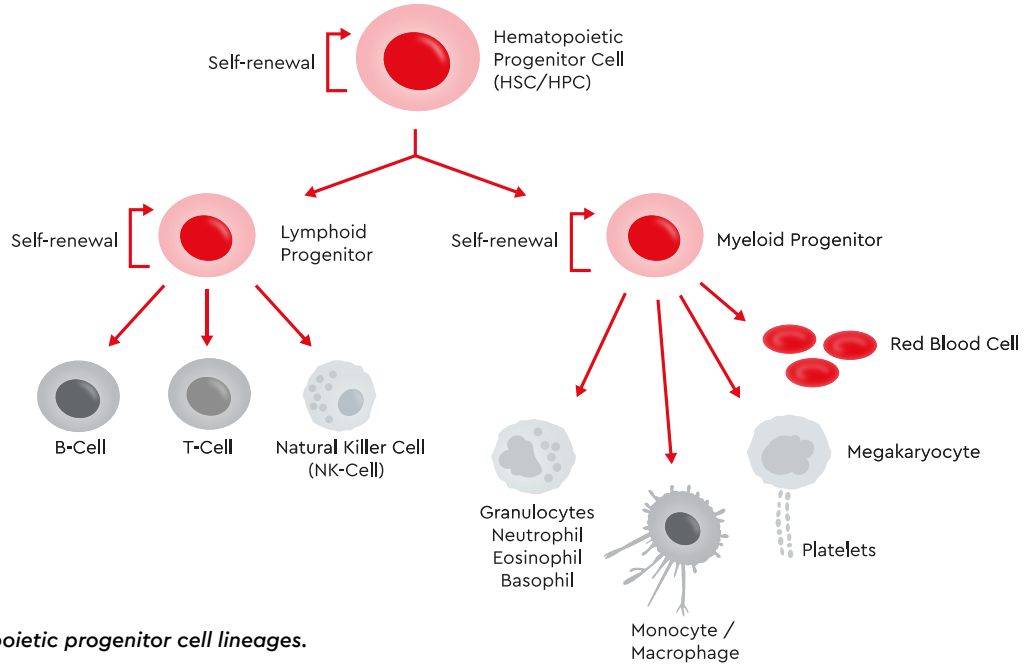

Fig. 1: Overview of hematopoietic progenitor cell lineages.

Protocol for expansion of primitive HPCs

I. Expansion Protocol of HSCs/HPCs

Materials

- HPCs, e.g. hCD34+-CB-c (C-12921) or freshly isolated HPCs
- Hematopoietic Progenitor Cell (HPC) Expansion Medium XF (C-28021)
- Appropriate cytokines, e.g., C-39890 or C-39891 (see below for details)
- Plasticware for suspension culture (non-TC treated)

Use aseptic techniques and a laminar flow bench.

1

Prepare the expansion medium

Combine the Basal Medium and the SupplementMix of the PromoCell HPC Expansion Medium XF (C-28021) according to the instructions.
Then, add an appropriate amount of PromoCell Cytokine Mix E to obtain the completely supplemented Expansion Medium.
Cytokine Mix E is a 100x concentrate: Cytokine Mix C-39890 (1 ml) is sufficient for the supplementation of 100 ml medium, while Cytokine Mix C-39891 (5 ml) is sufficient for 500 ml medium. Alternatively, you may supplement the medium with cytokines of your choice.

Note: Our HPC Expansion Medium XF must be supplemented with appropriate cytokines in order to successfully expand HPCs. The combination of the Basal Medium and SupplementMix is not sufficient.

After addition of Cytokine Mix E, the medium is stable for two weeks, if stored protected from light at 2–8°C. In daily routine, prewarm the necessary amount of the supplemented medium only.

2

Seed the HPCs (Day 0)

Freshly Isolated HPCs
Plate the HPCs at a density of 20,000 cells per ml in cytokine-supplemented complete medium (see step 1) using a suspension culture vessel.

Cryopreserved HPCs
Thaw the cells for two minutes in a waterbath (refer to the instruction manual for Human CD34+ Progenitor Cells C-12921 for details). Use at least 9 ml of complete medium per vial of cryopreserved cells (10 ml total volume per vial of thawed cells). Count the cells. Then, centrifuge the sample for 10 minutes at 240 x g at room temperature. Aspirate the supernatant, resuspend the cell pellet at 20,000 cells/ml complete medium and plate them in an appropriate suspension culture vessel.

Note: In order to avoid the accidental aspiration of the almost invisible HPC pellet after centrifugation, aspirate the supernatant gently and leave a residue of 100–200 µl in the tube.

3

Add fresh medium to the HPCs (Day 2–3)

Incubate the cells for two to three days at 37°C and 5% CO_2. Then, double the media volume of the sample by simply adding fresh complete medium and continue the incubation of the cells.

Note: Do not perform a medium change, just add fresh medium.

4

Expand the HPCs (Day 3+)

Incubate the cells for an additional 10–12 days at 37°C and 5% CO_2 by performing a partial medium change every two to three days, e.g., Monday, Wednesday and Friday:
For a partial medium change, remove the cells from the incubator. To create a single cell suspension gently pipet up and down several times and transfer the whole content of the culture vessel into an appropriate centrifugation tube. Pellet the cells by centrifugation at room temperature for 10 minutes at 240 x g. Then, discard half of the volume of the supernatant and gently resuspend the cells in the remaining volume of the medium. Add the resuspended cells to three times the volume of fresh complete medium in an appropriate suspension culture vessel. Repeat this partial media change every two to three days till the end of the expansion phase.

Example: For a partial media change of a culture volume of 8 ml, after centrifugation 4 ml of the supernatant are discarded and the cells are resuspended in the remaining 4 ml of the supernatant. Then these 4 ml of cell suspension are added to 12 ml of fresh complete medium.

5

Harvest expanded HPCs (Day 12+)

Harvest cells by collecting the medium from the tissue culture vessel containing the expanded HPC. Gently pipet up and down several times in order to release loosely attached cells and to obtain a single cell suspension. Spin down the harvested HPC at 240 g for 10 minutes and discard the supernatant.

Note: Harvesting of the cells should be performed within 48 hours after the last partial media change.

6

Resuspend and count the cells

Resuspend the cells in complete HPC Expansion Medium XF or an appropriate buffer of your choice and count them.

Note: : In general, depending on the donor a total expansion factor of approximately 300–1000 fold and an expansion of the $CD34^+$ population by approximately 50–200 fold can be expected when using the Cytokine Mix E.

The HPC are now ready to be used in your experiments, e.g., further enrichment steps or CFC-formation assays.

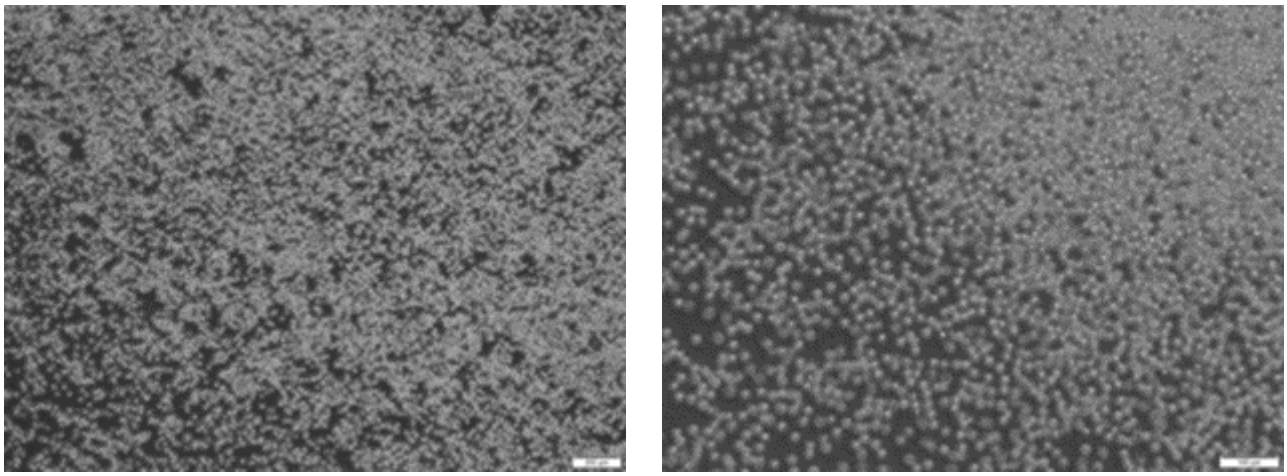

Fig. 2: Representative phase contrast images of the expansion of cryopreserved cord blood CD34⁺ cells (C-12921) using the HPC Expansion Medium XF (C-28021) supplemented with the Cytokine Mix E (C-39890).
Twenty thousand CD34⁺ cells per ml were expanded for 14 days according to the expansion protocol. The images were taken after the first half of the expansion phase on day seven. Left image: 40x magnification, right image: 100x magnification.

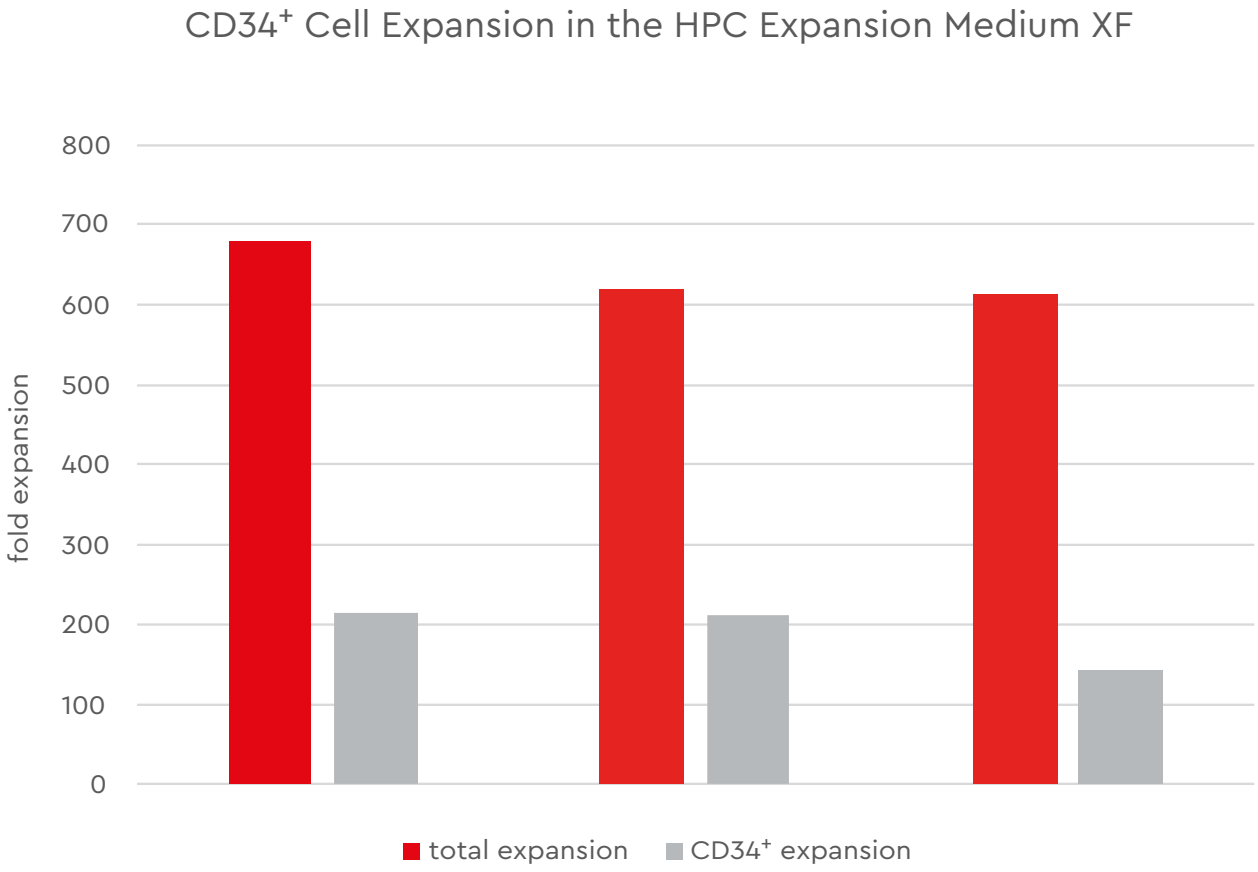

Fig. 3: Representative data of the expansion of three different donors of cryopreserved cord blood CD34⁺ cells (C-12921) using the HPC Expansion Medium XF (C-28021) supplemented with the Cytokine Mix E (C-39890).
Twenty thousand cells per ml were expanded for 12 days according to the given expansion protocol. The total expansion performance as well as the expansion factor of the CD34⁺ fraction of the obtained cell population was analyzed.

References

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4. Thomas ED, Lochte HL, Lu WC and Ferrebee JW: Intravenous Infusion of Bone Marrow in Patients Receiving Radiation and Chemotherapy. N Engl J Med (1957), 257: 491–6
5. Sauvageau G, Iscove NN and Humphries RK: *In vitro* and *in vivo* expansion of hematopoietic stem cells. Oncogene (2004), 23: 7223–7232

Related Products

Product	Size	Catalog Number
Hematopoietic Progenitor Cell Expansion Medium XF	500 ml	C-28021
Cytokine Mix E for HPC Expansion Medium XF	1 ml (sufficient for 100 ml Medium) 5 ml (sufficient for 500 ml Medium)	C-39890 C-39891
Lymphocyte Separation Medium 1077	500 ml	C-44010
Human CD34+ Progenitor Cells from Cord Blood (hCD34+-CB), single donor	100,000 cryopreserved cells	C-12921

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