Expansion of primitive Hematopoietic Progenitor Cells

PromoCell

Application Note

Background

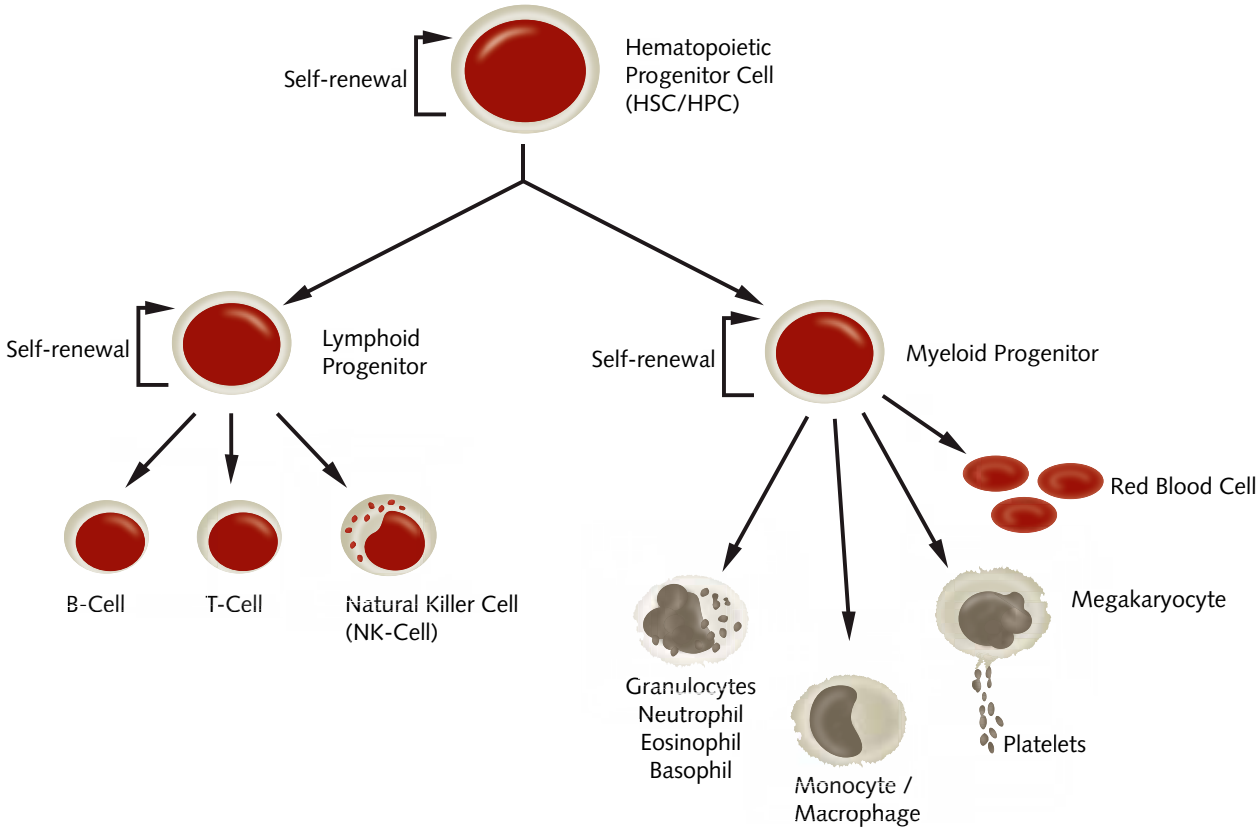
Hematopoietic Stem Cells (HSC/HPC) represent a heterogeneous population of primitive blood progenitor cells that fall into several different classes [1,2]. Within the human body, they are mainly located in the bone marrow of adults, but are also found in various fetal tissues, e.g. umbilical cord blood,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

HSC 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-lymphocytes, B-lymphocytes and NK-cells [3].

Treatments using HSC, e.g. bone marrow transplantation, have been used for over 40 years now and are well-established [4]. Indeed, HSC 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* HSC are able to expand to very large numbers of daughter cells by virtue of their pronounced self-renewal abilities [5]. However, to date researchers faced difficulties during *in vitro* expansion of undifferentiated HSC, with increasing differentiation of the cells observed during the expansion phase in traditional serum-containing culture systems. The establishment of serum-free, however still ill-defined formulations provided a somewhat more consistent culture environment, but the results were still not acceptable. Indeed, only a completely defined, well-supplemented growth medium seems adequate for the robust expansion of primitive multipotent hematopoietic cells.

PromoCell HPC Expansion Medium DXF (Cat. No. C-28021) provides a highly optimized, chemically defined and xeno-free culture system for human HSC, e.g. $CD34^+$ and $CD133^+$ cells. Optimal support of self-renewal and inhibition of differentiation result in superior expansion performance for human hematopoietic progenitor cells.



Use aseptic techniques and a laminar flow bench.

Expansion of undifferentiated HSC/HPC using HPC Expansion Medium DXF

Expansion of primitive HPC

1. Prepare the Expansion Medium

Combine the Basal Medium and the SupplementMix of the PromoCell HPC Expansion Medium DXF (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:
Cat. No. C-39890, 1 ml is sufficient for the supplementation of 100 ml Medium
Cat. No. C-39891, 5 ml is sufficient for the supplementation of 500 ml Medium
Alternatively the user may supplement the medium with cytokines of his choice.

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

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

2. Seed HPC

Pre-equilibrate an appropriate amount of the supplemented medium in the incubator at 37°C and 5% CO_2 for 30 minutes.

Freshly isolated HPC
Plate the HPC in the pre-equilibrated medium at a density of 5,000 cells per ml.

Cryopreserved HPC
Thaw the cells for 2 minutes in a waterbath (refer to the PromoCell Instruction Manual for Human CD34$^+$ Progenitor Cells for details). After thawing, immediately transfer them into the pre-equilibrated medium at a density of 5,000 cells per ml. Use at least 9 ml of medium per vial of cryopreserved cells.
Leave the cells untouched in an incubator at 37°C and 5% CO_2 for 4 – 8 hours. Then, perform a complete medium change as described in the subcultivation section of the PromoCell Instruction Manual for Human CD34$^+$ Progenitor Cells. Briefly, spin the cells down at 240 x g for 10 minutes, aspirate the supernatant and resuspend the cell pellet in fresh medium.

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. Expand the undifferentiated HPC

Incubate the cells for 4 days at 37°C and 5% CO_2.

For a partial medium change, remove the cells from the incubator. To create a single cell suspension pipet up and down several times and transfer the whole content of the tissue culture vessel into a 50 ml conical tube. Spin the cells down for 10 min at 240 x g. Then, carefully aspirate the upper two thirds of the medium. Gently resuspend the cells in the remaining third of the medium and replenish to the original volume with fresh cytokine-supplemented HPC Expansion Medium DXF.

Incubate for another 6 – 8 days to allow sufficient expansion of the cells. Replace two thirds of the medium as described above every 3 days.

4. Harvest expanded HPC

Harvest cells by collecting the medium from the tissue culture vessel containing the expanded HPC. 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 x g for 10 minutes and discard the supernatant.

5. Resuspend and count the cells

Resuspend the cells in PromoCell HPC Expansion Medium DXF and count them.

Note: The yield of primitive HSC after expansion depends on donor variance and the type of hematopoietic stem cell used. In general, a 75 – 300 fold expansion of the $CD34^+/CD38^-$ population can be expected. In addition, 5 – 20% of more differentiated cells may be observed.

The HPC are now ready to be used in your experiments, e.g. the differentiation into lineages of mature blood cells.
The non-directed differentiation of Hematopoietic Cells into mature blood cells can be achieved using the PromoCell Hematopoietic Progenitor Medium (C-28020). In order to direct the differentiation process into specific lineages of mature blood cells, the PromoCell Hematopoietic Progenitor Medium may be supplemented with appropriate cytokines by the user.

6. Characterize the cells (optional)

Characterize the hematopoietic immuno-phenotype of the expanded hematopoietic stem cells, e.g. by flow cytometry analysis. Primitive HPC exhibit a $CD34^+/CD38^-$ phenotype (see Fig. 2) and stain moderately positive for CD45.

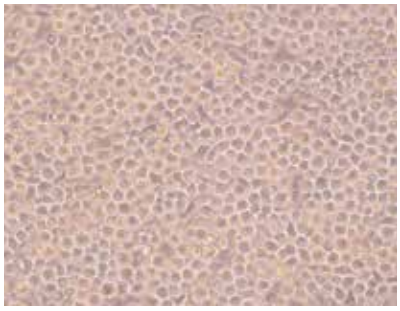


Fig. 1: Primitive Hematopoietic Progenitor Cells (HPC) exhibit strong growth in PromoCell´s Hematopoietic Progenitor Cell Expansion Medium DXF.

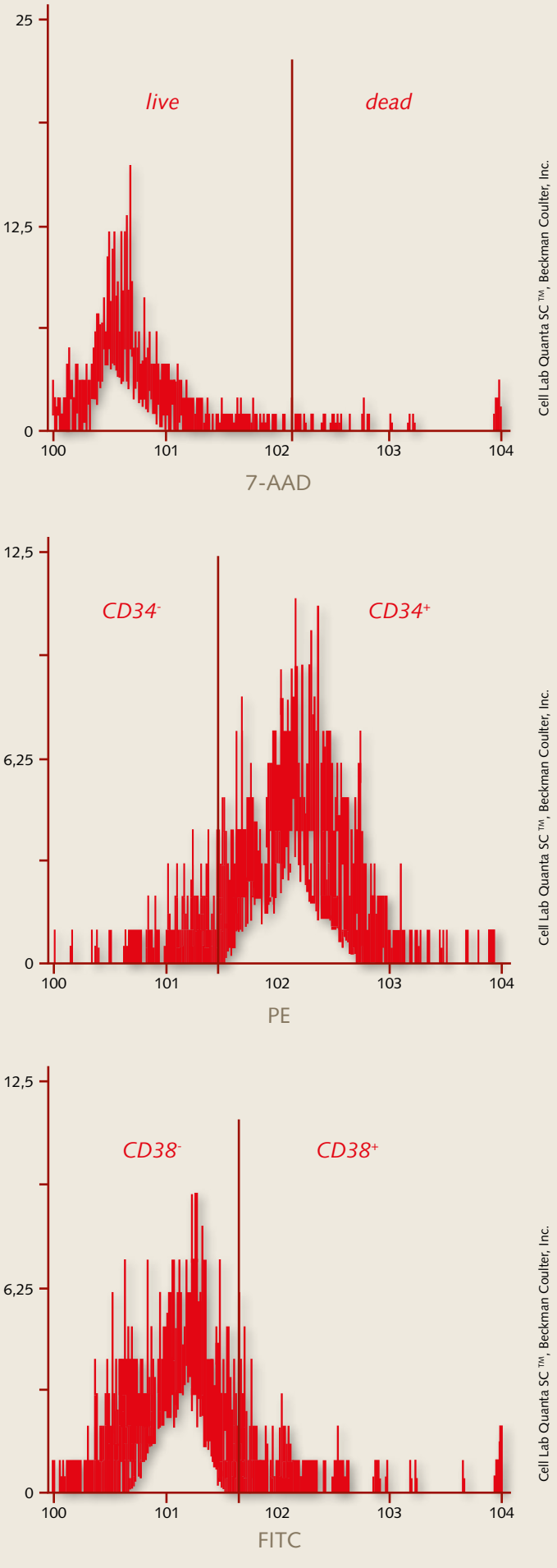


Fig. 2: Flow cytometry analysis of cord blood-derived CD34⁺ cells after 12 days of expansion in PromoCell HPC Expansion Medium DXF. More than 98% of the cells are viable after the expansion period as detected by 7-AAD staining (A). The percentage of cells staining positive for CD34 is >93% (B), while >95% of the cells do not stain for CD38 (C). The CD34⁺/CD38⁻ marker profile indicates the maintenance of a primitive hematopoietic phenotype.

References

[1] Sieburg HB, Cho RH, Dykstra B, Eaves, CJ, Muller-Sieburg, CE. The hematopoietic stem cell compartment consists of a limited number of discrete stem cell subsets. Blood. 2006, 107: 2311-6

[2] Schroeder, T. Hematopoietic Stem Cell Heterogeneity: Subtypes, Not Unpredictable Behavior. Cell Stem Cell 2010, 6(3): 202-7

[3] Metcalf D, Concise Review: Hematopoietic Stem Cells and Tissue Stem Cells: Current Concepts and Unanswered Questions. STEM CELLS 2007, 25: 2390–5

[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 DXF	500 ml	C-28021
Cytokine Mix E for HPC Expansion Medium DXF	1 ml (sufficient for 100 ml Medium) 5 ml (sufficient for 500 ml Medium)	C-39890 C-39891
Hematopoietic Progenitor Medium (Ready-to-use)	100 ml	C-28020
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
TPO, human, recombinant *(E. coli)*	10 µg	C-65112
IL-3, human, recombinant	10 µg	C-61320
IL-6, human, recombinant	20 µg	C-61625
flt-3 Ligand, human, recombinant *(E. coli)*	10 µg	C-67110
EPO-alpha, human, recombinant	50 µg	C-60022
SCF, human, recombinant *(E. coli)*	10 µg	C-63120
G-CSF, human, recombinant *(E. coli)*	10 µg	C-60434

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

Email: info@promocell.com
www.promocell.com

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 90 93 32 (ligne verte)
Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

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