Expansion of primitive hematopoietic progenitor cells

PromoCell®

Application note

Background

Hematopoietic stem cells (HSCs)/hematopoietic progenitor cells (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 found in adult bone marrow but are also present in various fetal tissues, including umbilical cord blood, the placenta, and the fetal liver.

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

Our HPC Expansion Medium XF is a highly optimized, serum free,and xeno-free culture system supporting robust expansion of human HPCs (e.g., $CD34^+$ and $CD133^+$ populations). It is available as:

- HPC Expansion Medium XF (C-28021
- HPC Expansion Medium XF, phenol red-free (C-28022)
- PromoExQ HPC Expansion Medium XF, phenol red-free (EQ-C-28022), our Excipient GMP-grade* quality product, manufactured according to the EXCiPACT™ GMP certification scheme for pharmaceutical excipients, ensuring the highest quality standards

It is known that *in vivo* HPCs are able to generate large numbers of daughter cells due to their high proliferative capacity, while long-term self-renewal is primarily retained by HSCs.[5] CD34 is widely used as a surface marker to identify and enrich primitive human hematopoietic stem and progenitor cell populations. It is commonly applied as a practical readout when characterizing or manufacturing HSCs/HPCs-containing grafts for transplantation and translational workflows.[6–8] However, researchers still face difficulties while expanding HSCs/HPCs in traditional serum-containing media, as the cells tend to differentiate under these conditions.[9,10]

The cell populations expanded using our HPC Expansion Medium XF are enriched with functional progenitor cells capable of colony-forming cell (CFC) formation in appropriate in vitro assays. The medium can be used with Cytokine Mix E, a ready-to-use growth factor cocktail containing recombinant human TPO, SCF, FLT-3 ligand, and IL-3, to support consistent expansion performance.

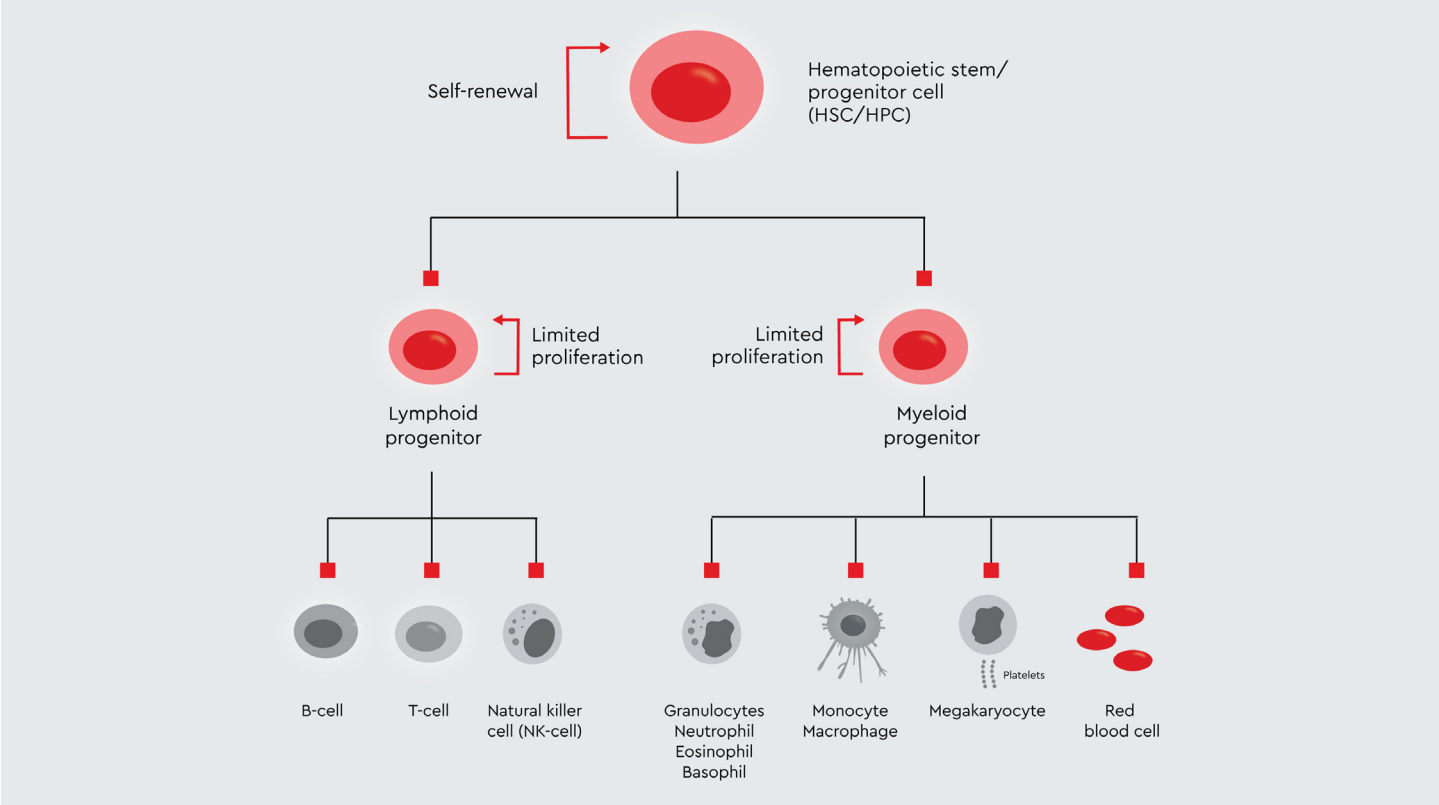


Fig. 1: *Overview of hematopoietic stem and progenitor cell lineages.*

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 (for self-isolation workflows, refer to the protocol for our Lymphocyte separation media)
- Hematopoietic Progenitor Cell (HPC) Expansion Medium XF (C-28021) or HPC Expansion Medium XF, phenol red-free (C-28022), or PromoExQ HPC Expansion Medium XF, phenol red-free (EQ-C-28022)
- Appropriate cytokines, e.g., C-39890 or C-39891 (see below for details)
- Plasticware for suspension culture (non-TC treated)

1

Prepare the expansion medium

Combine the Basal Medium and the SupplementMix of the PromoCell HPC Expansion Medium XF (C-28021) with or without phenol red 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 to successfully expand HPCs. The combination of the Basal Medium and SupplementMix is not sufficient. Cytokine Mix E contains recombinant human TPO, SCF, FLT-3 ligand, and IL-3 for optimal growth conditions. After the addition of Cytokine Mix E, the medium remains stable for 2 weeks when stored protected from light at 2–8°C. In daily routine, prewarm only the necessary amount of supplemented medium.

2

Seed the HPCs

Freshly isolated HPCs
Plate the HPCs at a density of 10,000 cells per ml in cytokine-supplemented complete medium (see step 1) using a suspension culture vessel.

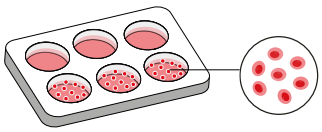
Cryopreserved HPCs
Thaw the cells for 2 minutes in a water bath (refer to the instruction manual for Human CD34+ Progenitor Cells C-12921 for details). Use at least 9 ml of complete medium per vial of cryopreserved cells (10 ml total volume per vial of thawed cells). Count the cells. Then, centrifuge the sample for 10 minutes at 240 x g at room temperature. Aspirate the supernatant, resuspend the cell pellet at 10,000 cells/ml in complete medium, and plate them in an appropriate suspension culture vessel. For a 6-well plate, a seeding volume of 2 ml per well is recommended to start the culture (20,000 cells per well). A detailed example of a cultivation workflow is provided at the end of this application note (see Table 2).

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.

Day	Procedure
0	Seed cells at 10,000 cells/ml (e.g., 6-well plate, 2 ml per well)
2 or 3	Add fresh medium (doubling the total culture volume; don't remove medium)
5–12	Perform partial medium changes every 2–3 days (after centrifugation, discard half of the supernatant, resuspend, and add 3x volume of fresh medium; scale up vessel as needed (e.g., transfer to T75 flask on Day 7 at 16 ml total volume)
13–15	Harvest cells (within 48 h of last partial medium change)

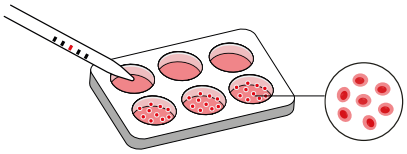
Table 1: *Overview of cultivation workflow*

Day 0: Start the culture



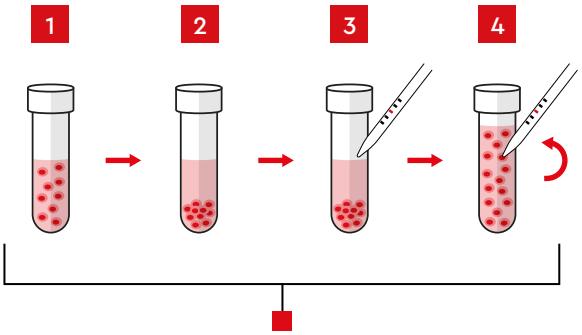
Seed cells: 10,000 cells/ml
2 ml per well (6-well plate)

Day 2–3: Add fresh medium



Add 2 ml fresh medium
Total volume: 4 ml per well

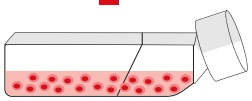
Days 5–12: Partial medium changes every 2–3 days



1. Collect cell suspension from culture vessel and transfer into a centrifugation tube
2. Pellet cells by centrifugation
3. Carefully remove half of the supernatant without disturbing the pellet
4. Gently resuspend cells and add 3x volume of fresh medium

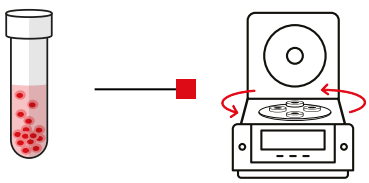
Repeat every 2–3 days

Return resuspended cells to a culture vessel to continue growth



Scale up as needed
Example: Day 7 – transfer to T75 flask (16 ml total volume)

Day 13–15: Harvest cells



Collect the suspension culture, centrifuge, and resuspend cells for counting

Cells are cultured in suspension (non-adherent conditions) in non-TC treated plasticware
Seeding density: 10,000 cells/ml | Centrifugation: 240 × g, 10 min, RT

Fig. 2: *Cultivation workflow for the expansion of human hematopoietic progenitor cells (HPCs).*

3

Add fresh medium (Day 2 or 3)

Incubate the cells for 2–3 days at 37°C and 5% CO_2. Then, add an equal volume of fresh complete medium to double the total culture media volume and continue incubation.

Note: Don't change or remove the medium at this step; simply add fresh medium.

4

Expand the HPCs (Days 5–12)

Incubate the cells for an additional 10–12 days at 37°C and 5% CO_2, performing a partial medium change every 2–3 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, then transfer the entire contents 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 supernatant volume and gently resuspend the cells in the remaining 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 2–3 days till the end of the expansion phase.

Example: If the culture volume is 8 ml, discard 4 mL after centrifugation. Resuspend the cells in the remaining 4 ml, then add to 12 ml fresh complete medium (final 16 ml).

5

Harvest expanded HPCs (Day 13–15)

Harvest the cells by collecting the medium from the tissue culture vessel containing the expanded HPCs. Gently pipet up and down several times to release loosely attached cells and to obtain a single-cell suspension. Spin down the harvested HPCs at 240 × g for 10 minutes, then 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: After approximately 2 weeks (13–15 days), depending on the donor, a total expansion factor of over 300-fold of total cell population, and an expansion of the $CD34^+$ population by approximately 50–200 fold can be expected when using the Cytokine Mix E. The HPCs are now ready to be used in your experiments (e.g., further enrichment steps or CFC-formation assays).

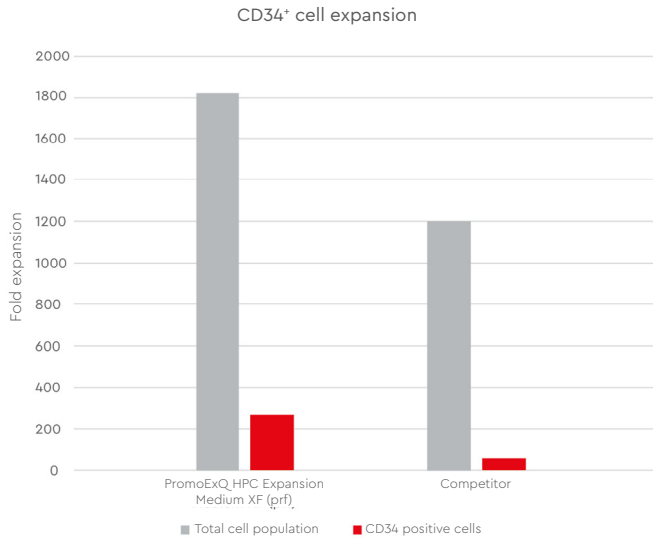


Fig. 3: Representative data of the expansion of cryopreserved human CD34+ cells. *Cells derived from cord blood after 13 days of culture in PromoExQ HPC Expansion Medium XF (prf) or competitor medium. Cells were seeded at 10,000 cells/ml and harvested on Day 13.*

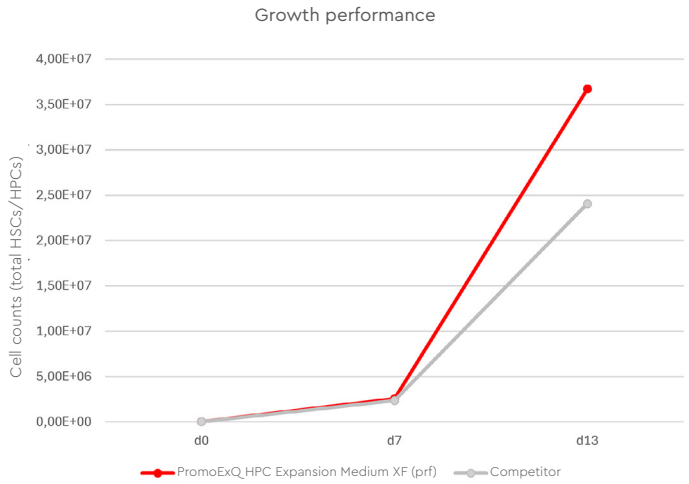


Fig. 4: Representative data of the growth kinetics of HSCs/HPCs cultured in PromoExQ HPC Expansion Medium XF (prf) or competitor medium. *Cells were initially seeded at 20,000 cells per well in a 6-well plate and, after expansion, harvested on Day 13. Amount of viable cells were determined by automated Cytek® Guava® Muse® cell analyzer (Cytek Biosciences).*

	Percentage of CD34 positive HSCs/HPCs		
	Day 0	Day 7 [Ø]	Day 13 [Ø]
PromoExQ HPC Expansion Medium XF (prf)	95 %	28 %	15 %
Competitor	95 %	11 %	5 %

Table 2: Medium comparison and representative data of the maintenance of CD34+ cell fraction of HSCs/HPCs by FACS analysis after approximately 2 weeks of culture. *Cells were seeded at 10,000 cells/ml and harvested on Day 13. Mean values of CD34 positive cells after cultivation in PromoExQ HPC Expansion Medium XF (prf) or competitor medium. The table shows exemplary data from analyzed samples in PromoExQ HPC Expansion Medium XF (prf) in a time dependent manner on day 0 (day of thawing), day 7 and day 13. As a negative control for FACS gating strategy unstained cells were used (left panel).*

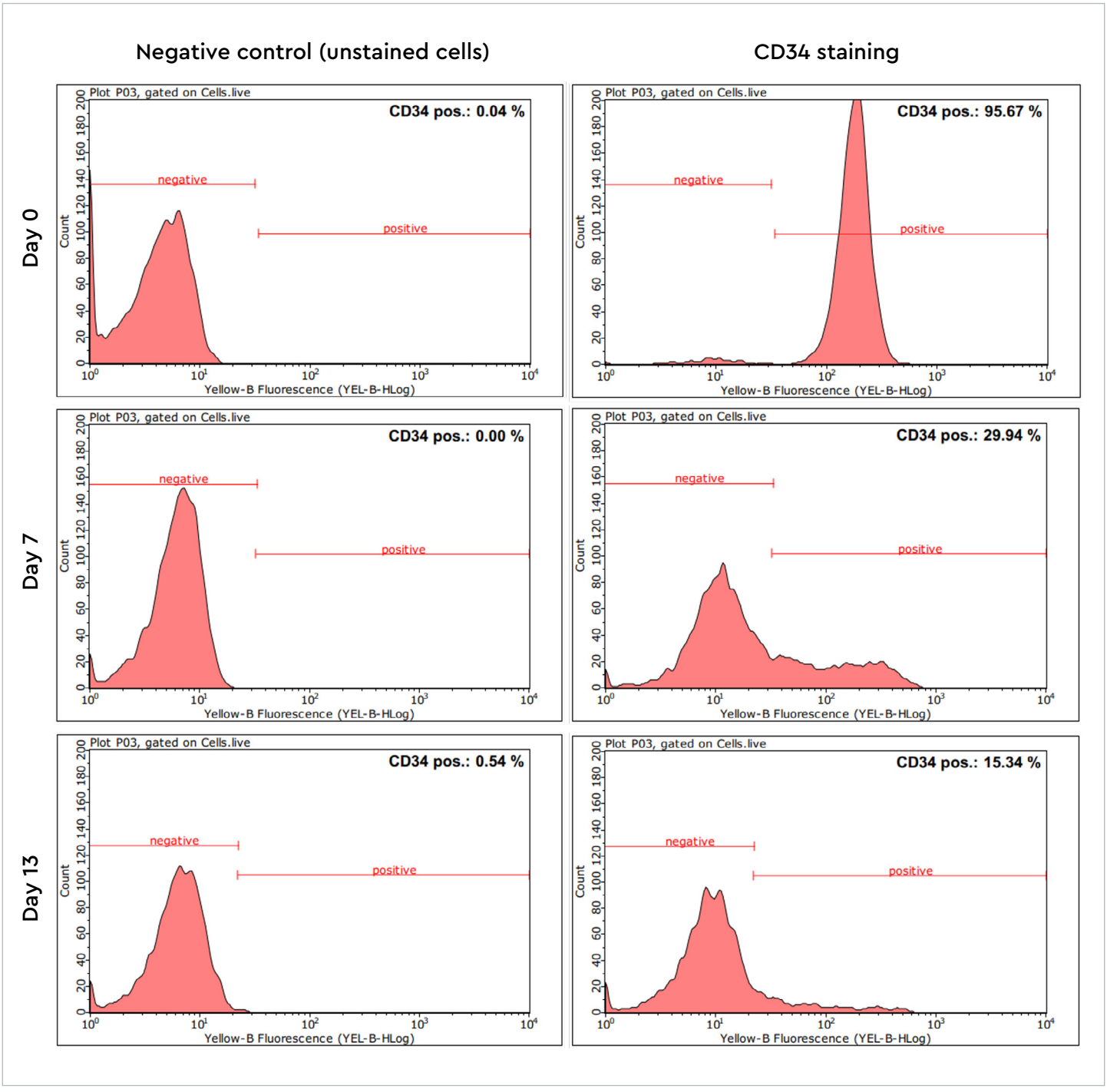


Fig. 5: Flow cytometric analysis of CD34 expression during expansion of human hematopoietic progenitor cells. *Cells were analyzed on Days 0, 7, and 13 of culture. Histograms show the fluorescence intensity of CD34 staining in cells gated on the live cell population (verified by 7AAD staining). The left panel shows unstained controls, and the right panel shows CD34-stained samples. The percentage of CD34⁺ cells in the gated population is shown in each plot. This analysis illustrates the change in the CD34⁺ cell fraction during expansion culture, while progenitor cells remain present throughout the cultivation period.*

Process	Weekday	Day after thawing	Time interval	Vessel	Medium added	Final medium volume
Thawing	Wednesday	Day 0	-	6-well	-	2 ml
Add fresh medium	Friday	Day 2	48 ± 5 h	6-well	2 ml	4 ml
1st medium change	Monday	Day 5	72 ± 5 h	6-well	6 ml	8 ml
2nd medium change	Wednesday	Day 7	48 ± 5 h	T75 flask	12 ml	16 ml
3rd medium change	Friday	Day 9	48 ± 5 h	T75 flask	24 ml	32 ml
4th medium change	Monday	Day 12	72 ± 5 h	T75 flask	48 ml	64 ml
Final cell count	Tuesday	Day 13	24 ± 5 h	T75 flask	-	64 ml

Table 3: *Cultivation workflow example (start with 6-well plate format)*

* *"GMP grade" is a branding term used by PromoCell to denote reagents that are manufactured at the PromoCell manufacturing facility in Heidelberg, Germany, under strictly controlled processes to meet stringent product specifications and customer requirements. Reagents manufactured at PromoCell are produced in accordance with EXCiPACT™ GMP standards, a quality management system that builds on our ISO 9001:2015 certification. Risk assessment procedures are carried out at the customer site.*

Materials and related products

Product	Size	Catalog number
Hematopoietic Progenitor Cell Expansion Medium XF	500 ml	C-28021
Hematopoietic Progenitor Cell Expansion Medium XF, phenol red free	500 ml	C-28022
PromoExQ Hematopoietic Progenitor Cell Expansion Medium XF, phenol red free	500 ml	EQ-C-28022
Cytokine Mix E for HPC Expansion Medium XF	1 ml (sufficient for 100 ml medium)	C-39890
Cytokine Mix E for HPC Expansion Medium XF	5 ml (sufficient for 500 ml medium)	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
Cryo-SFM Plus	30 ml/125 ml	C-29920/C-29922
Cryo-SFM Plus, phenol red-free	30 ml/125 ml	C-29930/C-29932

Contact us

Need technical guidance or support with your experimental design? Get in touch with our scientific support team.

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PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

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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