# CD34+ Progenitor Cells

PromoCell®

## Instruction manual

| Product | Size | Catalog number |
|---|---|---|
| Human CD34+ Progenitor Cells from Cord Blood (hCD34+-CB) single donor | 100,000 cryopreserved cells | C-12921 |

### Product description

CD34 is a glycosylated transmembrane protein and represents a well-known marker for primitive blood- and bone marrow-derived progenitor cells, especially for hematopoietic and endothelial progenitors. Although the biological functions of CD34 are largely unknown, recent data suggest that CD34 is involved in maintenance of the progenitor cells in a phenotypically undifferentiated state. We offer CD34+ Progenitor Cells from the cord blood of healthy donors, produced at our cell culture facility. CD34+ Progenitor Cells are suitable for a series of studies, e.g. directed differentiation into more committed types of blood cells and endothelial lineages.

Shortly after isolation, all our Human CD34+ Progenitor Cells are cryopreserved using our proprietary, animal-component free, and protein-free cryopreservation medium (for details, please refer to the Certificate of Analysis). Each cryovial contains more than 100,000 viable cells after thawing.

### Quality control

Rigid quality control tests are performed for each lot of our CD34+ Progenitor Cells.

They are routinely characterized by flow cytometry analyzing the viability, cell size, and the presence of significant markers, i.e. CD34.

In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV, HCV, HTLV-1, HTLV-2 and microbial contaminants (fungi, bacteria, and mycoplasma).

### Intended use

PromoCell CD34+ Progenitor Cells are for *in vitro* research use only and not for diagnostic or therapeutic procedures.

### Warning

Although tested negative for HIV-1, HIV-2, HBV, HCV, HTLV-1 and HTLV-2,the cells – like all products of human origin – should be handled as potentially infectious. No test procedure can completely guarantee the absence of infectious agents.

*Follow appropriate safety precautions!*

> *On delivery, immediately follow the protocol for cryopreserved cells (see page 2).*

# Instructions for cryopreserved Human $CD34^+$ Progenitor Cells

## Materials

- hCD34+-CB-c (C-12921)
- 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.*

# I. Expansion protocol

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## 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. Prewarm the medium or an aliqot for 30 minutes at room temperature before seeding the cells.

**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.

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## Seed the HPCs (Day 0)

Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice – even for short transportation. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then retighten. Immerse the vial into a water bath (37°C) up to the height of the screw cap for 2 minutes. Ensure that no water enters the thread of the screw cap. Thoroughly rinse the cryovial with 70% ethanol under a laminar flow bench. Then, aspirate the excess ethanol from the thread area of the screw cap. Open the vial and transfer the cells to a centrifugation tube containing prewarmed, complete expansion medium from step 1. 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.

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## 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.

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## 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.

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## 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 x 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.

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## 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.

## Specifications

| Product | Recommended culture media | Plating density |
|---|---|---|
| Human CD34+ Progenitor Cells from Cord Blood (hCD34+-CB), single donor | C-28021<br>C-39891 | 20,000 cells per ml |

## 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)<br>5 ml (sufficient for 500 ml Medium) | C-39890<br>C-39891 |
| Cryo-SFM Plus | 30 ml<br>125 ml | C-29920<br>C-29922 |

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10/2024