

StemSpan™ CC100

Serum-free culture supplement for expansion of human hematopoietic cells

Catalog #02690 1 mL



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Product Description

StemSpan™ CC100 cytokine cocktail contains a combination of both early- and late-acting recombinant human (rh) cytokines formulated to support the proliferation of human hematopoietic cells. It is supplied as a 100X concentrate.

When added to serum-free medium, StemSpan™ CC100 promotes the expansion of CD34+ cells isolated from human cord blood (CB), bone marrow (BM), or mobilized peripheral blood (mPB), and is recommended if high cell yields and large numbers of CD34+ cells are desired.

We recommend using StemSpan™ CC100 in combination with any of the following StemSpan™ media:

- StemSpan™ SFEM (Catalog #09600)
- StemSpan™ SFEM II (Catalog #09605)
- StemSpan™-XF (Catalog #100-0073)
- StemSpan™-AOF (Catalog #100-0130)

Properties

Storage: Store at -20° to -70°C.

Shelf Life: Stable until expiry date (EXP) on label.

Contains:

- Recombinant human fms-like tyrosine kinase 3 ligand (Flt3L)
- Recombinant human stem cell factor (SCF)
- Recombinant human interleukin 3 (IL-3)
- Recombinant human interleukin 6 (IL-6)

Directions for Use

PREPARATION OF COMPLETE MEDIUM

1. Thaw StemSpan™ CC100 at room temperature (15 - 25°C) or at 37°C until just thawed. Mix thoroughly.

NOTE: If necessary, centrifuge for 30 seconds to remove liquid from cap.

NOTE: If not used immediately, store at 2 - 8°C for up to 1 month. Alternatively, aliquot and store at -20°C. After thawing aliquots, store at 2 - 8°C for up to 1 month; do not re-freeze. Do not exceed the shelf life of the supplement.

2. Add StemSpan™ CC100 to culture medium at a 1 in 100 dilution (e.g. add 1 mL of supplement to 99 mL of culture medium). Mix thoroughly.

RECOMMENDED PROTOCOL FOR CD34+ CELL EXPANSION WITH StemSpan™ MEDIA

For optimal performance, use one of the StemSpan™ media suggested below.

1. Prepare StemSpan™ medium of choice by following instructions in the applicable Product Information Sheet, available at www.stemcell.com, or contact us to request a copy.
 - StemSpan™ SFEM II (Document #10000000430)
 - StemSpan™-AOF (Document #10000016677)

NOTE: If using StemSpan™ SFEM II, ensure the medium is completely thawed and mixed thoroughly before use.

2. Prepare complete medium as described in the Preparation section, using a StemSpan™ medium as the culture medium.

OPTIONAL: Add UM729 (Catalog #72332) to a final concentration of 1 µM; titration may be required to determine the optimal concentration for CD34+ cell expansion. UM729 enhances expansion of CD34+ cells, in addition to more primitive subsets such as CD34+CD90+CD45RA- cells.

3. Thaw cryopreserved CD34+ cells or use an EasySep™ kit to isolate CD34+ cells from fresh whole CB, BM, mPB, or frozen mononuclear cells (MNCs), as indicated in Table 1.

Alternatively, source frozen isolated CD34+ cells from BM (e.g. Human Bone Marrow CD34+ Cells, Frozen, Catalog #70002*), CB (e.g. Human Cord Blood CD34+ Cells, Frozen, Catalog #70008*), or mPB (e.g. Mobilized Human Peripheral Blood CD34+ Cells, Frozen, Catalog #70060*).

Table 1. Recommended Cell Isolation Kits for Various Cell Sources

CELL SOURCE	RECOMMENDED CELL ISOLATION KIT
Fresh whole CB	EasySep™ Human Cord Blood CD34 Positive Selection Kit II (Catalog #17896)
Fresh BM (e.g. Human Whole Bone Marrow, Fresh, Catalog #70502*)	EasySep™ Human CD34 Positive Selection Kit II (Catalog #17856)
Fresh peripheral blood mobilized with granulocyte colony-stimulating factor (G-CSF), plerixafor, or a combination of both (e.g. Human Mobilized Peripheral Blood Leukopak, G-CSF, Fresh, Catalog #200-0602*)	EasySep™ Human CD34 Positive Selection Kit II (Catalog #100-1569)
Frozen MNCs from BM (e.g. Human Bone Marrow Mononuclear Cells, Frozen, Catalog #70001*), CB (e.g. Human Cord Blood Mononuclear Cells, Frozen, Catalog #70007*), or mPB (e.g. G-CSF Mobilized Human Peripheral Blood Mononuclear Cells, Frozen, Catalog #70049*)	EasySep™ Human CD34 Positive Selection Kit II (Catalog #17856)

* Some primary cell products are available only in select regions. Contact us at techsupport@stemcell.com for further information.

4. **Day 0:** Plate CD34+ cells in complete medium with or without UM729 (prepared in step 2). Refer to Table 2 for recommended plating concentrations; optimal cell concentrations and cultureware are dependent on experimental objectives and cell quality.

Table 2. Recommended CD34+ Cell Concentrations for Various Cultureware

CULTUREWARE*	VOLUME OF MEDIUM PER WELL	NUMBER OF CELLS PER WELL
6-well plate	2 mL	2 x 10 ⁴
24-well plate	1 mL	1 x 10 ⁴
96-well plate	100 µL	1 x 10 ³

*Both tissue culture-treated and non-tissue culture-treated are suitable.

5. Incubate at 37°C and 5% CO₂.
6. **Day 3 or 4:** Add an equal volume of fresh complete medium or perform a half-medium change.
7. **Day 7:** Harvest cells for evaluation or downstream applications. Count total viable cells using Trypan Blue (e.g. Catalog #07050) and a hemocytometer (e.g. Catalog #100-1181), or an automated cell counting method, and measure CD34+ expression by flow cytometry. Additional immunophenotyping may be performed to identify CD34+ cell subsets and/or differentiated CD34- cells.

NOTE: A 7-day culture period is optimal for cell yield, CD34 expression, and progenitor cell function. Shorter culture periods of 24 - 72 hours may be used if preserving stem and progenitor cell function is desired over cell yield. Culturing beyond 7 days can be considered if high cell yields are desired. However, CD34 expression and progenitor cell function is reduced with longer culture times, due to cell differentiation.

If culturing for > 7 days: Cultures can be continued for an extended period of time with periodic dilution every 3 - 4 days to maintain a cell concentration < 1 x 10⁵ cells/mL.

NOTE: Antigen expression on cultured cells may not be as predictive for determining non-differentiated status or lineage potential compared to antigen expression on CD34+ cells that have not been cultured. For example, primary CD34+ cells with low or undetectable CD38 expression (CD34+CD38- phenotype) are highly enriched for hematopoietic stem cells and primitive progenitor cells, but CD34+CD38- phenotype of cultured cells may not be as primitive.

Notes and Tips

ASSESSMENT OF HEMATOPOIETIC CELLS

Assessment of CD34+ cells before and after culture may be performed by flow cytometry using the following fluorochrome-conjugated antibody clones:

- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013) or Clone 563 (Catalog #60119) or Clone 8G12 (Catalog #60121), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018) or Clone 2D1 (Catalog #60123), and
- Anti-Human CD38 Antibody, Clone AT-1 (Catalog #100-1578) or Clone HIT2 (Catalog #60014), and
- Anti-Human CD90 Antibody, Clone 5E10 (Catalog #60045)

Related Products

For related products, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com/HSPCworkflow, or contact us at techsupport@stemcell.com. For available fresh and cryopreserved peripheral blood, cord blood, and bone marrow products in your region, visit www.stemcell.com/primarycells.

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