

# 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) and bone marrow (BM), 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°C 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, do not re-freeze.

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 following StemSpan™ media in this protocol:

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

1. Thaw StemSpan™ medium at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

NOTE: If not used immediately, aliquot into tubes and store at -20°C. Once aliquots are thawed, do not re-freeze.

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 or BM, or from cryopreserved CB or BM mononuclear cells (MNCs), as indicated below:
  - For fresh whole CB, use EasySep™ Human Cord Blood CD34 Positive Selection Kit II (Catalog #17896)
  - For fresh BM, or frozen BM or CB MNCs, use EasySep™ Human CD34 Positive Selection Kit II (Catalog #17856)
4. **Day 0:** Plate CD34+ cells in complete medium with or without UM729 (prepared in step 2). Refer to Table 1 for recommended plating concentrations; optimal cell concentrations and cultureware are dependent on experimental objectives and cell quality.

**Table 1. Recommended CD34+ Cell Concentrations for Various Cultureware**

CULTUREWARE*	VOLUME OF MEDIUM PER WELL	NUMBER OF CELLS PER WELL
6-well plate	2 mL	$2 \times 10^4$
24-well plate	1 mL	$1 \times 10^4$
96-well plate	100 $\mu$ L	$1 \times 10^3$

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

5. Incubate at 37°C and 5% CO<sub>2</sub>.
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 (Catalog #07050) and a hemocytometer (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 CD34 expression and progenitor cell function is desired over high 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 <  $1 \times 10^5$  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.

## 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)
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018) or Clone 2D1 (Catalog #60123)
- Anti-Human CD38 Antibody, Clone AT-1 (Catalog #60131) or Clone HIT2 (Catalog #60014)
- Anti-Human CD90 Antibody, Clone 5E10 (Catalog #60045)

## Notes and Tips

### RELATED PRODUCTS

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

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