

# StemSpan™ HSC Plus Supplement

**Animal origin-free supplement for the expansion of human hematopoietic stem and progenitor cells**

Catalog #100-1694 1 mL



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## Product Description

StemSpan HSC Plus Supplement contains a combination of small molecules formulated to selectively promote the expansion of human CD34+ hematopoietic stem and progenitor cells (HSPCs) or purified CD34+ subsets enriched for hematopoietic stem cells (HSCs), when added to culture medium containing appropriate cytokines.

### Advantages:

- Formulated to selectively expand primitive HSPCs, including CD34+CD45RA-CD90+CD133+EPCR+ cells in liquid cultures initiated with CD34+ cells or purified CD34+ subsets isolated from cord blood (CB), mobilized peripheral blood (mPB), or bone marrow (BM).
- Optimized for use with StemSpan™ media and StemSpan™ cytokine supplements.
- Supplied as a 100X concentrate. After thawing and brief vortexing, the tube content can be added directly to the hematopoietic cell expansion medium.

## Properties

**Storage:** Store at -20°C.

**Shelf Life:** Stable for 12 months from date of manufacture (MFG) on label.

## Preparation of Reagents and Materials

### A. StemSpan™ HSC PLUS SUPPLEMENT

Thaw StemSpan™ HSC Plus Supplement at room temperature (15 - 25°C). Mix thoroughly. Do not thaw at 37°C as this may cause precipitation.

NOTE: If not used immediately, store at 2 - 8°C for up to 1 week. 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.

### B. COMPLETE MEDIUM

For optimal performance, use one of the StemSpan™ media suggested below. Alternatively, a culture medium of your choice can be used.

1. Prepare StemSpan™ medium of choice by following instructions in the applicable Product Information Sheet, available at [www.stemcell.com](http://www.stemcell.com), or contact us to request a copy.

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

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

2. Add one of the following supplements to the culture medium:

- StemSpan™ CD34+ Expansion Supplement (10X; Catalog #02691) at a 1 in 10 dilution
- StemSpan™ CC100 (Catalog #02690) or StemSpan™ CC110 (Catalog #02697) at a 1 in 100 dilution
- Cytokines of choice

3. Add StemSpan™ HSC Plus Supplement (prepared in section A) to the culture medium at a 1 in 100 dilution. Mix thoroughly.

## Directions for Use

### HSPCs EXPANSION OF PURIFIED CD34+ CELLS

1. Prepare complete medium as described in Preparation of Reagents and Materials, section B.
2. Thaw cryopreserved CD34+ cells, or use an EasySep™ kit to isolate CD34+ cells from fresh whole CB, mPB, or BM, or from cryopreserved CB, mPB, or BM 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, Fresh, G-CSF, 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](mailto:techsupport@stemcell.com) for further information.

3. **Day 0:** Plate CD34+ cells in complete medium. 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/ WELL	NUMBER OF CD34+ CELLS/ WELL
6-well plate	1 mL	1 x 10 <sup>4</sup>
24-well plate	0.5 mL	5 x 10 <sup>3</sup>
96-well plate	100 µL	1 x 10 <sup>3</sup>

4. Incubate cells at 37°C and 5% CO<sub>2</sub>.
5. **Day 4:** Add an equal volume of fresh complete medium.
6. **Day 7:** Harvest cells for evaluation or downstream applications. Count total viable cells using Trypan Blue (Catalog #07050) and a hemocytometer (e.g. Catalog #100-1181) or an automated cell counting method. Measure the expression for HSC-specific cell surface markers by flow cytometry.

NOTE: A 7-day culture period is optimal for HSPCs expansion. Shorter culture periods of 24 - 72 hours may be used if preserving stemness and progenitor cell function is preferred. Culturing beyond 7 days can be considered if high cell yields are desired. However, the frequency of primitive HSPCs is reduced with longer culture times, due to cell differentiation.

7. **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 of < 1 x 10<sup>6</sup> cells/mL.

## Assessment of Hematopoietic Cells

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

- Anti-human CD34 Antibody, clone 561 (BioLegend Catalog #343610)
- Anti-human CD45RA Antibody, Clone HI100 (BioLegend Catalog #304106)
- Anti-human CD90 Antibody, Clone 5E10 (Catalog #60045AZ.1)
- Anti-human CD201 (EPCR) Antibody, Clone RCR-401 (BioLegend Catalog #351904)
- Anti-human CD133/1 Antibody, Clone AC133 (Miltenyi Biotec Catalog #130-113-110)
- Anti-human CD38 Antibody, Clone HIT2 (BioLegend Catalog #303522)

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.

## Related Products

For related products, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit [www.stemcell.com](http://www.stemcell.com), or contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com).

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