StemSpan™ HSC Plus Supplement

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

Catalog #100-1694 1 mL

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

StemSpan™ HSC Plus Supplement contains a combination of small molecules formulated to selectively promote the expansion of hematopoietic stem and progenitor cells (HSPCs) in cultures of human CD34+ cells or purified CD34+ subsets enriched for hematopoietic stem cells (HSCs), when added to culture medium containing appropriate cytokines. StemSpan™ HSC Plus Supplement is supplied as a 100X concentrate.

- 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
- 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: Once thawed, use immediately or store at 2 - 8°C for up to 1 week. Alternatively, aliquot and store at -20°C. After thawing aliquots, do not re-freeze.

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, 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 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 10^4
24-well plate	0.5 mL	5 x 10^3
96-well plate	100 µL	1 x 10^3

4. Incubate cells at 37°C and 5% CO_2.
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 10^6 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, or contact us at techsupport@stemcell.com.