

# StemSpan™ SFEM

Serum-free medium for culture and expansion of hematopoietic cells

Catalog #09600      100 mL  
#09650              500 mL



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## Product Description

StemSpan™ Serum-Free Expansion Medium (SFEM) has been developed and tested for the in vitro culture and expansion of human hematopoietic cells, when the appropriate growth factors and supplements are added. This allows users the flexibility to prepare medium that meets their requirements. When combined with the appropriate cytokines, StemSpan™ SFEM has been used for the culture and expansion of hematopoietic cells isolated from other species, including mouse, non-human primate, and dog (Bauer et al.; Miller & Eaves; Sandrin et al.; Zhang & Lodish). StemSpan™ SFEM has also been used for culture of various other hematopoietic and non-hematopoietic cell types. Using appropriate StemSpan™ Expansion Supplements, StemSpan™ SFEM may be used to expand CD34+ cells isolated from human cord blood (CB), mobilized peripheral blood (mPB), or bone marrow (BM) samples, or to expand and differentiate lineage-committed progenitors to generate populations of erythroid, myeloid, or megakaryocyte progenitor cells.

StemSpan™ SFEM II (Catalog #09605) is an improved version of StemSpan™ SFEM that is further enriched to promote and support higher rates of CD34+ expansion and/or cell differentiation.

## Properties

- Storage:** Store at -20°C.
- Shelf Life:** Stable until expiry date (EXP) on label.
- Contains:**
- Iscove's MDM
  - Bovine serum albumin
  - Recombinant human insulin
  - Human transferrin (iron-saturated)
  - 2-Mercaptoethanol
  - Supplements

This product contains material derived from human plasma. Donors have been tested and found negative for hepatitis B surface antigen (HBsAg) and HIV-1 antibodies and/or HIV-1 antigen. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

## Directions for Use

### PREPARATION OF COMPLETE MEDIUM

1. Thaw StemSpan™ SFEM at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.  
NOTE: If not used immediately, store at 2 - 8°C for up to 1 month. Alternatively, aliquot into tubes and store at -20°C. After thawing aliquots, do not re-freeze. Do not exceed the shelf life of the medium.
2. Add desired cytokines, growth factors, and other components to StemSpan™ SFEM. Mix thoroughly.  
NOTE: Added components and cells in sterile cell culture medium (e.g. Iscove's MDM or DMEM) should not exceed ~10% of total volume.
3. Add cells, mix thoroughly, and set up cultures as desired.

## RECOMMENDED PROTOCOL FOR CD34+ CELL EXPANSION WITH StemSpan™ SFEM

For a complete list of expansion supplements for use with StemSpan™ SFEM, see Notes and Tips. Refer to the Product Information Sheet (PIS) for each supplement for recommended cell expansion protocols with StemSpan™ media.

For instructions to expand CD34+ cells using StemSpan™ SFEM and StemSpan™ CD34+ Expansion Supplement (10X; Catalog #02691), see below.

1. Prepare StemSpan™ SFEM as described in the Preparation section on page 1.

OPTIONAL: Add UM729 (Catalog #72332) to a final concentration of 1  $\mu$ M; 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.

2. 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.

3. **Day 0:** Plate CD34+ cells in complete medium with or without UM729. 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	$2 \times 10^4$
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.

4. Incubate at 37°C and 5% CO<sub>2</sub>.
5. **Day 3 or 4:** Add an equal volume of fresh complete medium or perform a half-medium change.
6. **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  $< 1 \times 10^5$  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.

## 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 #60131) or Clone HIT2 (Catalog #60014), and
- Anti-Human CD90 Antibody, Clone 5E10 (Catalog #60045)

STEMCELL Technologies recommends the use of Human LDL (Catalog #02698) as a culture supplement. It has been pre-screened for the culture, expansion, and colony assay of human hematopoietic and non-hematopoietic cells in serum-free culture media. It promotes the proliferation and survival of human hematopoietic and other progenitor cells in culture, resulting in increased cell output in expansion cultures and increased colony numbers and/or colony size in colony assays. Selection of an optimal growth factor combination is dependent upon the source and type of cells and the experimental objectives of the researcher. StemSpan™ expansion supplements, described below, are suitable for use with StemSpan™ SFEM.

- StemSpan™ CD34+ Expansion Supplement (10X) (Catalog #02691)
  - Culture and expansion of large numbers of human CD34+ progenitor cells
  - Contains: rh SCF, rh TPO, rh IL-3, rh IL-6, rh Flt3 ligand, other additives
- StemSpan™ CC100 (Catalog #02690)
  - Culture and expansion of human hematopoietic cells
  - Contains: rh Flt3 ligand, rh SCF, rh IL-3, rh IL-6
- StemSpan™ CC110 (Catalog #02697)
  - Culture and expansion of human hematopoietic cells
  - Contains: rh Flt3 ligand, rh SCF, rh TPO
- StemSpan™ Erythroid Expansion Supplement (100X) (Catalog #02692)
  - Expansion and lineage-specific differentiation of human CD34+ cells into erythroid progenitor cells
  - Contains: rh SCF, rh IL-3, rh EPO
- StemSpan™ Megakaryocyte Expansion Supplement (100X) (Catalog #02696)
  - Expansion and lineage-specific differentiation of human CD34+ cells into megakaryocyte progenitor cells
  - Contains: rh SCF, rh TPO, rh IL-6, rh IL-9
- StemSpan™ Myeloid Expansion Supplement (100X) (Catalog #02693)
  - Expansion and lineage-specific differentiation of human CD34+ cells into granulocytes
  - Contains: rh SCF, rh TPO, rh G-CSF, rh GM-CSF
- StemSpan™ Myeloid Expansion Supplement II (100X) (Catalog #02694)
  - Expansion and lineage-specific differentiation of human CD34+ cells into monocytes
  - Contains: rh Flt3 ligand, rh SCF, rh TPO, rh M-CSF, rh GM-CSF, other additives

SCF = stem cell factor; EPO = erythropoietin; TPO = thrombopoietin; rh = recombinant human; IL = interleukin; Flt = fms-like tyrosine kinase

## 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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