

StemSpan™ SFEM II

Serum-free medium for culture and expansion of hematopoietic cells

Catalog #09605 100 mL
#09655 500 mL



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TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713

INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM

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Product Description

StemSpan™ Serum-Free Expansion Medium II (SFEM II) is a modified version of StemSpan™ SFEM. It has been developed for the in vitro culture and expansion of human hematopoietic cells. This medium contains pre-tested bovine serum albumin, insulin, transferrin, and other supplements in Iscove's MDM. Recombinant hematopoietic growth factors, required for the optimal growth and expansion of hematopoietic cells, have not been added to StemSpan™ SFEM II. This allows users the flexibility to prepare medium that meets their requirements.

Using appropriate cytokines (e.g. StemSpan™ CC100, StemSpan™ CC110, or StemSpan™ CD34+ Expansion Supplement; see Notes and Tips), StemSpan™ SFEM II can be used for the expansion of total nucleated cells and CD34+ cells from cord blood (CB), bone marrow (BM), or other cell sources. StemSpan™ SFEM II can also be used to expand and differentiate lineage-committed progenitor cells to generate erythroblasts, granulocytes, monocytes, or megakaryocytes when used with StemSpan™ Erythroid Expansion Supplement (Catalog #02692), StemSpan™ Myeloid Expansion Supplement (Catalog #02693), StemSpan™ Myeloid Expansion Supplement II (Catalog #02694), or StemSpan™ Megakaryocyte Expansion Supplement (Catalog #02696), respectively.

Properties

- Storage:** Store at -20°C.
- Shelf Life:** Stable for 18 months from date of manufacture (MFG) 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 II 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 II. 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.
NOTE: Addition of lipids is not recommended.

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

For a complete list of expansion supplements for use with StemSpan™ SFEM II, 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 II and StemSpan™ CD34+ Expansion Supplement (10X; Catalog #02691), see below.

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

OPTIONAL: Add UM729 (Catalog #72332) to a final concentration of 1 μ 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, mobilized peripheral blood (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 | 2×10^4 |
| 24-well plate | 1 mL | 1×10^4 |
| 96-well plate | 100 μ L | 1×10^3 |

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

4. Incubate at 37°C and 5% CO₂.
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)

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 II.

- 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 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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