StemSpan™ Erythroid Expansion Supplement (100X)

Serum-free culture supplement for expansion of human erythroid cells

Catalog #02692 1 mL

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Product Description

StemSpan™ Erythroid Expansion Supplement (100X) contains a combination of recombinant human cytokines formulated to selectively promote the expansion and differentiation of erythroid progenitor cells from CD34+ cells isolated from human cord blood (CB), bone marrow (BM), or mobilized peripheral blood (mPB) samples.

StemSpan™ Erythroid Expansion Supplement (100X) is intended for use in combination with any of the following StemSpan™ media:

- StemSpan™ SFEM (Catalog #09600)
- StemSpan™ SFEM II (Catalog #09605)
- StemSpan™-XF (Catalog #100-0073)
- StemSpan™-ACF Erythroid Expansion Medium (Catalog #09860)

Advantages:

- Formulated to produce large numbers of human erythroid cells in liquid cultures initiated with CD34+ cells isolated from CB, BM, or mPB cells.
- Optimized for use with StemSpan™ media. When combined with StemSpan™ SFEM II in particular, supports up to 4-fold higher expansion of erythroid cells from human CD34+ CB cells than other serum-free media on the market.
- Supplied as a 100X concentrate. After thawing and mixing, the tube content can be added directly to any hematopoietic cell expansion medium of choice.

Properties

Storage: Store at -20 to -70°C.

Shelf Life: Stable until expiry date (EXP) on label.

Contains:

- Recombinant human stem cell factor (SCF)
- Recombinant human interleukin 3 (IL-3)
- Recombinant human erythropoietin (EPO)

Directions for Use

PREPARATION OF COMPLETE MEDIUM

1. Thaw StemSpan™ Erythroid Expansion Supplement (100X) at room temperature (15 - 25°C) until just thawed. Mix thoroughly.

   NOTE: If necessary, centrifuge for 30 seconds to recover liquid from cap.
2. NOTE: If not used immediately, store supplement at 2 - 8°C for up to 1 month. 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.
3. Add StemSpan™ Erythroid Expansion Supplement (100X) 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 ERYTHROID PROGENITOR CELL EXPANSION WITH StemSpan™ MEDIA

For optimal performance, use one of the StemSpan™ media suggested below.

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 II (Document #10000000430)
   - StemSpan™-ACF Erythroid Expansion Medium (Document #10000005419)

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

2. Prepare complete medium as described in the Preparation section, using a StemSpan™ medium as the culture medium.

   OPTIONAL: Supplement medium with glucocorticoid receptor agonist (1 µM dexamethasone or hydrocortisone) to inhibit erythroblast maturation (generates more pro-erythroblasts, fewer normoblasts, and has similar overall erythroid cell yields).

3. 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.

4. Day 0: Plate CD34+ cells in complete medium at 1 x 10^4 cells/mL. 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 x 10^4
24-well plate	1 mL	1 x 10^4
96-well plate	100 µL	1 x 10^3

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

5. Incubate at 37°C and 5% CO_2.
6. Day 3 or 4: Add an equal volume of fresh complete medium (i.e. if using a 24-well plate, add 1 mL of complete medium per well).
7. Day 7 and 10 or Day 11: Harvest cells and replate in fresh complete medium at < 1 x 10^5 cells/mL; greater expansion may be achieved if a lower density is used (e.g. 2 x 10^4 cells/mL). Alternatively, add complete medium to maintain desired cell concentration.
8. Day 14: 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 erythroid progenitor cell surface marker expression by flow cytometry.

   NOTE: Cultures can be continued beyond 14 days with periodic dilution every 3 - 4 days to maintain a cell concentration of 1 x 10^5 cells/mL.

Notes and Tips

ASSESSMENT OF DIFFERENTIATED CELLS

Assessment of hematopoietic stem and progenitor cells before and after culture, and erythroid cells 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 CD235a (Glycophorin A) Antibody, Clone 2B7 (Catalog #60152), and
- Anti-Human CD71 (Transferrin Receptor) Antibody, Clone OKT9 (Catalog #60106)

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.