

# 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) or bone marrow (BM) 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+ CB or BM 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 other serum-free media on the market.
- Supplied as a 100X concentrate. After thawing and mixing, the tube contents can be added directly to any hematopoietic cell expansion medium of choice.

## Properties

**Storage:** Store at -20°C 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.  
NOTE: Once thawed, store supplement at 2 - 8°C for up to 1 month. Alternatively, aliquot and store at -20°C. After thawing aliquots, do not re-freeze.
2. 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 following StemSpan™ media in this protocol:

- StemSpan™ SFEM II (Catalog #09605)
- StemSpan™-ACF Erythroid Expansion Medium (Catalog #09860)

1. Thaw StemSpan™ medium at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.  
NOTE: If not used immediately, aliquot into tubes and store at -20°C. Once aliquots are thawed, do not re-freeze.
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 or BM, or from cryopreserved CB or BM mononuclear cells (MNCs), as indicated below:
  - For fresh whole CB, use EasySep™ Human Cord Blood CD34 Positive Selection Kit II (Catalog #17896)
  - For fresh BM, or frozen BM or CB MNCs, use EasySep™ Human CD34 Positive Selection Kit II (Catalog #17856)
4. **Day 0:** Plate CD34+ cells in complete medium at  $1 \times 10^4$  cells/mL. Refer to Table 1 for recommended plating concentrations; optimal cell concentrations and cultureware are dependent on experimental objectives and cell quality.

**Table 1. 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.

5. Incubate at 37°C and 5% CO<sub>2</sub>.
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 Day 10 or 11:** Harvest cells and replate in fresh complete medium at  $< 1 \times 10^5$  cells/mL; greater expansion may be achieved if a lower density is used (e.g.  $2 \times 10^4$  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 (Catalog #07050) and a hemocytometer (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  $1 \times 10^5$  cells/mL.

## 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)
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018) or Clone 2D1 (Catalog #60123)
- Anti-Human CD235a (Glycophorin A) Antibody, Clone 2B7 (Catalog #60152)
- Anti-Human CD71 (Transferrin Receptor) Antibody, Clone OKT9 (Catalog #60106)

## Notes and Tips

### 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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