

# StemSpan™ Megakaryocyte Expansion Supplement (100X)



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Catalog #02696

1 mL

## Product Description

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

StemSpan™ Megakaryocyte 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™-AOF (Catalog #100-0130)

### Advantages:

- Formulated to produce large numbers of human megakaryocytes in liquid cultures initiated with CD34+ CB or BM cells.
- Optimized for use with StemSpan™ media. When combined with SFEM II in particular, supports up to 2-fold higher expansion of megakaryocytes from human CD34+ CB cells than 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 6 (IL-6)
- Recombinant human interleukin 9 (IL-9)
- Recombinant human thrombopoietin (TPO)

## Directions for Use

### PREPARATION OF COMPLETE MEDIUM

1. Thaw StemSpan™ Megakaryocyte 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™ Megakaryocyte 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.  
NOTE: If desired, supplement the medium with 20 - 40 µg/mL Human LDL (Catalog #02698).

### RECOMMENDED PROTOCOL FOR MEGAKARYOCYTE 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](http://www.stemcell.com), or contact us to request a copy.
  - StemSpan™ SFEM II (Document #10000000430)
  - StemSpan™-AOF (Document #10000016677)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.
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  $1 \times 10^4$  to  $2 \times 10^4$  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	$2 \times 10^4$
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 7:** Harvest and replate in fresh complete medium at  $< 1 \times 10^5$  cells/mL. Alternatively, add fresh complete medium to maintain cell concentration at  $< 1 \times 10^5$  cells/mL.
7. **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 megakaryocyte 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.

## 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. 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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