

StemSpan™ Myeloid Expansion Supplement II (100X)



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Catalog #02694

1 mL

Product Description

StemSpan™ Myeloid Expansion Supplement II (100X) contains a combination of recombinant human cytokines formulated to selectively promote the expansion and differentiation of human myeloid progenitor cells, generating large numbers of CD14+ monocytes from CD34+ cells isolated from human cord blood (CB) samples.

StemSpan™ Myeloid Expansion Supplement II (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:

- Promotes the production of hundreds of CD14+ monocytes per input CD34+ human CB cell in 14-day liquid cultures.
- Supplied as a 100X concentrate. After thawing and mixing, the tube contents can be added directly to any hematopoietic cell expansion medium.

Properties

Storage: Store at -20°C.

Shelf Life: Stable for 15 months from date of manufacture (MFG) on label.

Contains:

- Recombinant human fms-like tyrosine kinase 3 ligand (Flt3L)
- Recombinant human stem cell factor (SCF)
- Recombinant human thrombopoietin (TPO)
- Recombinant human macrophage colony-stimulating factor (M-CSF)
- Recombinant human granulocyte/macrophage colony-stimulating factor (GM-CSF)
- Supplements

Directions for Use

PREPARATION OF COMPLETE MEDIUM

1. Thaw StemSpan™ Myeloid Expansion Supplement II (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™ Myeloid Expansion Supplement II (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 MONOCYTE EXPANSION WITH StemSpan™ MEDIA

For optimal performance, use StemSpan™ SFEM II medium.

1. Thaw StemSpan™ SFEM II medium at room temperature (15 - 25°C) or overnight at 2 - 8°C. Ensure the medium is completely thawed and mixed thoroughly before use.

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™ SFEM II medium as the culture medium.

3. Thaw cryopreserved CD34+ cells, or use an EasySep™ kit to isolate CD34+ cells from fresh whole CB or cryopreserved CB mononuclear cells (MNCs), as indicated in Table 1.
Alternatively, source frozen isolated CD34+ cells from CB (e.g. Human Cord Blood CD34+ Cells, Frozen, Catalog #70008*).

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)
Frozen MNCs from CB (e.g. Human Cord Blood Mononuclear Cells, Frozen, Catalog #70007*),	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 1×10^4 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	2×10^4
24-well plate	1 mL	1×10^4
96-well plate	100 µL	1×10^3

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

5. Incubate at 37°C and 5% CO₂.
6. **Day 3 or 4:** Add an equal volume of fresh complete medium (i.e. if using a 24-well plate, add 1 mL complete medium per well).
7. **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.
8. **Day 10/11:** Add an equal volume of fresh complete medium (i.e. if using a 24-well plate, add 1 mL complete medium per well) to maintain cell concentration at $< 1 \times 10^5$ cells/mL.
9. **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 monocyte 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 1×10^5 cells/mL.

Related Products

For related products, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com/HSPCworkflow 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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