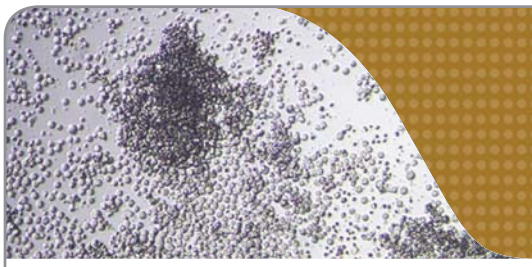

StemSpan™

Megakaryocyte Expansion Supplement (100X)

Background

Investigators studying hematopoiesis require standardized culture media and cytokines to promote the proliferation and/or lineage-specific differentiation of hematopoietic stem and progenitor cells (HSPC) from bone marrow (BM), cord blood (CB) and other tissues. STEMCELL Technologies has developed a family of expansion media, which includes serum-free and animal component-free formulations (see page 2). StemSpan™ media require the addition of cytokines (eg. StemSpan™ Expansion Supplements) to promote HSPC proliferation and differentiation. The choice of cytokines and supplements is dependent on the objective of the experiment, i.e. on the desired numbers of specific cell types to be generated in vitro.

Product Description

StemSpan™ Megakaryocyte Expansion Supplement (100X) (Catalog #02696; formerly known as CC220) contains a combination of recombinant human cytokines (SCF, IL-6, IL-9 and TPO) and other additives formulated to selectively promote the expansion and differentiation of megakaryocyte progenitor cells in cultures of BM-, CB- and mobilized peripheral blood-derived CD34$^+$ cells. It is optimized for use in combination with StemSpan™ SFEM, SFEM II and -ACF media.

Data

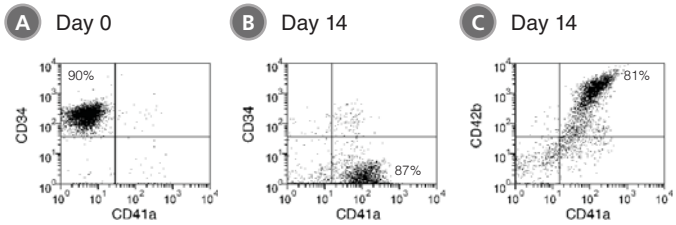

Figure 1. Production of Megakaryocytes by the Expansion and Lineage-Specific Differentiation of CB-Derived CD34$^+$ Cells Cultured in StemSpan™ SFEM Containing the Megakaryocyte Expansion Supplement

Flow cytometry dot plots showing expression of the HSPC marker CD34 and megakaryocyte markers CD41a and CD42b (A) before and (B,C) after culture of CD34$^+$ CB cells for 14 days in StemSpan™ SFEM containing the Megakaryocyte Expansion Supplement. The frequency of CD34$^+$ cells declined from 90% before culture to < 3% after 14 days, in parallel with a gradual accumulation of CD41a$^+$CD42b$^+$ megakaryocytes from < 1 % to > 80% before and after culture, respectively.

Advantages:

- Defined and serum-free
- Promotes the production of hundreds of megakaryocytes per input human CB-derived CD34$^+$ cell in 14-day liquid cultures
- Optimized for use with StemSpan™ media

Table 1. Production of Megakaryocytes from CB-Derived CD34$^+$ Cells Cultured in StemSpan™ SFEM Containing Megakaryocyte Expansion Supplement

CB SAMPLE	CD41a$^+$ CELLS PRODUCED PER INPUT CD34$^+$ CELL	% CD41a$^+$ CELLS
1	240	93
2	80	89
3	360	90
4	330	89
5	190	89
6	60	76
Mean 95% CL*	210 80-340	88 81-94

Shown are the numbers and percentages of CD41a$^+$ cells produced per input CB-derived CD34$^+$ cell after 14 days of culture (n = 6).
*95% confidence limits (CL); the range within which 95% of the results will typically fall.

Applications:

- Research into the regulation of megakaryopoiesis
- Development of procedures to expand megakaryocytes and platelets in culture
- Assessment of effects of candidate therapeutics on megakaryocytes for drug development

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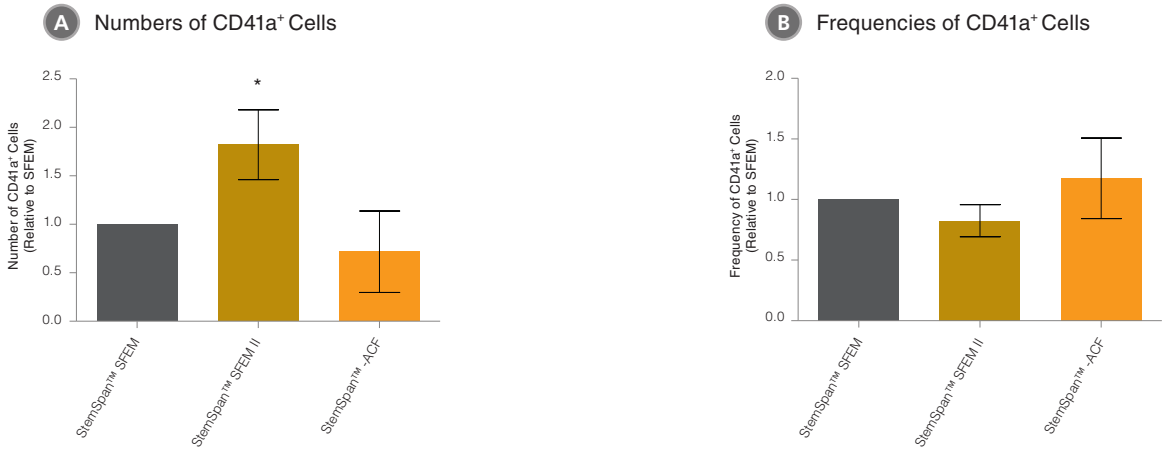

Figure 2. Comparison of Megakaryocyte Expansion in Different StemSpan™ Media Containing Megakaryocyte Expansion Supplement

(A) Average numbers and (B) frequencies of CD41a+ megakaryocytes normalized relative to the values obtained in StemSpan™ SFEM (grey bars) after culturing purified CB-derived CD34+ cells for 14 days in StemSpan™ SFEM, SFEM II (gold bars) or -ACF (orange bars) media containing Megakaryocyte Expansion Supplement. Vertical lines indicate 95% confidence limits, the range within which 95% of results typically fall. The numbers of CD41a+ cells were significantly higher in SFEM II, when compared to SFEM and -ACF medium (*$p < 0.01$; paired t-test, $n = 6$).

Media and Supplements for Megakaryocyte Expansion

PRODUCT	DESCRIPTION	RECOMMENDED FOR
StemSpan™ SFEM 09600 (100 mL) 09650 (500 mL)	Serum-free expansion medium (SFEM) containing pre-tested bovine serum albumin, insulin, transferrin and supplements in Iscove's MDM	Serum-free culture of human HSPCs
StemSpan™ SFEM II 09605 (100 mL) 09655 (500 mL)	Enhanced version of StemSpan™ SFEM containing pre-tested bovine serum albumin, insulin, transferrin and supplements in Iscove's MDM	Serum-free expansion of human HSPCs
StemSpan™-ACF 09805 (100 mL) 09855 (500 mL)	Animal component-free (ACF) medium containing only recombinant and synthetic components	Culture of human HSPCs in the absence of non-human animal-derived components
StemSpan™ Megakaryocyte Expansion Supplement (100x) (formerly CC220) 02696 (1 mL)	Pre-mixed cocktail of recombinant human cytokines (SCF, TPO, IL-6, IL-9)	Generation of megakaryocytes by expansion and lineage-specific differentiation of human hematopoietic progenitor cells

For related products for HSPC research, 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**.