

StemSpan™

Erythroid Expansion Supplement (100X)

Background

Investigators studying hematopoiesis require standardized culture media and cytokines to promote the proliferation and/or 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 addition of cytokines 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™ Erythroid Expansion Supplement (100X) (Catalog #02692) contains a combination of recombinant human cytokines (SCF, IL-3 and EPO) formulated to selectively promote the expansion and differentiation of erythroid progenitor cells in cultures of purified CD34⁺ cells from human CB, BM and mobilized peripheral blood. It is optimized for use in combination with StemSpan™ SFEM, SFEM II and ACF media.

Representative Results

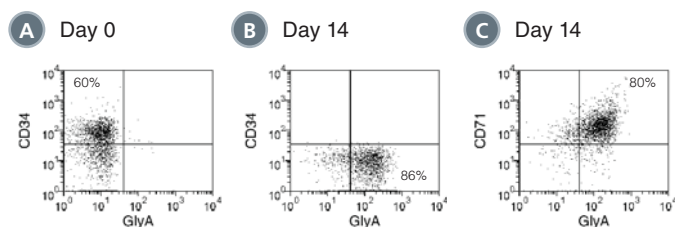


Figure 1. Production of erythroblasts by expansion and lineage-specific differentiation of CD34⁺ human cord blood cells cultured in StemSpan™ SFEM containing Erythroid Expansion Supplement.

Flow cytometry dot plots showing expression of the HSPC marker CD34 and erythroid markers CD71 and glycophorin-A (GlyA) (A) before culture and (B,C) after 14 days of culture of enriched CD34⁺ CB cells in StemSpan™ SFEM containing Erythroid Expansion Supplement. The frequency of CD34⁺ cells declined from ~60% before culture to <0.1% after 14 days. In parallel, erythroid cells gradually accumulated from levels of <1% before culture to >90% by day 14. The bulk of cell population recovered from 14-day culture consisted of CD71⁺GlyA⁺ erythroblasts. More immature CD71⁺GlyA⁻ progenitors and pro-erythroblasts, as well as more differentiated CD71^{-low} GlyA⁺ normoblasts were also present at low frequencies.

Advantages:

- Defined and serum-free
- Promotes the production of thousands of erythroid cells per input CD34⁺ human CB cell in 14-day liquid cultures
- Optimized for use with StemSpan™ media

CB SAMPLE	ERYTHROID CELLS PRODUCED PER INPUT CD34 ⁺ CELL	% ERYTHROID CELLS
1	1640	99
2	1620	99
3	1180	99
4	900	88
5	10130	71
6	10660	81
7	3120	80
8	2760	86
9	942	76
10	1170	78
11	200	54
12	6450	94
13	1420	66
14	4650	71
Mean	3350	82
95% CL*	1370-5320	74-90

Table 1. Production of erythroid cells from CD34⁺ human cord blood cells cultured in StemSpan™ SFEM containing Erythroid Expansion Supplement.

Numbers and percent of erythroid cells produced after 14 days of culture of enriched CD34⁺ cells from 14 different CB samples.

*95% confidence limits; the range within which 95% of the results will typically fall.



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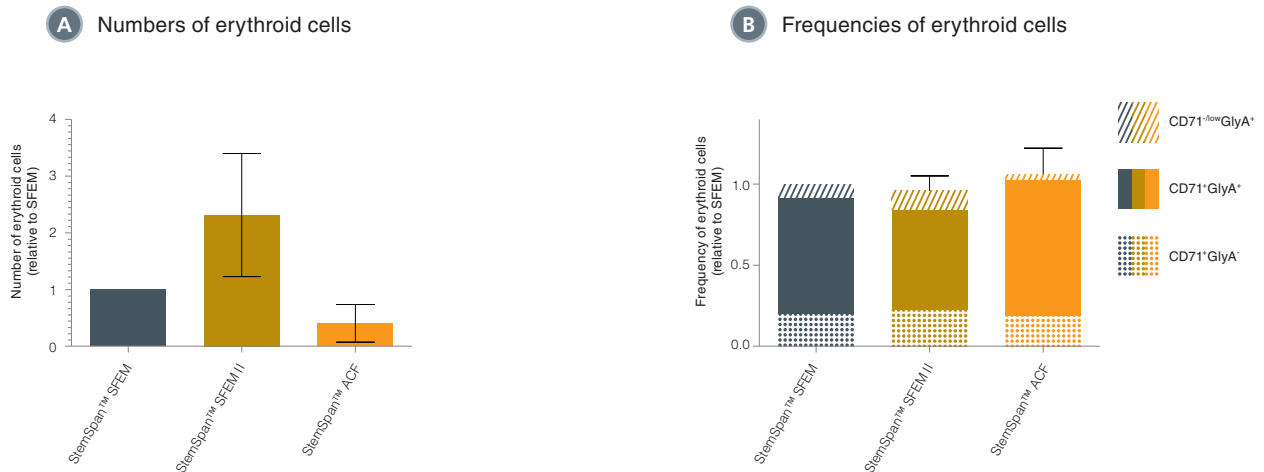


Figure 2. Comparison of erythroid cell expansion in different StemSpan™ media containing Erythroid Expansion Supplement.

(A) Average numbers and (B) frequencies of erythroid cells normalized relative to the values obtained in StemSpan™ SFEM (grey bars) after culturing purified CD34⁺ CB cells (n=6) for 14 days in StemSpan™ SFEM, SFEM II (gold bars) or ACF (orange bars) media containing Erythroid Expansion Supplement. Vertical lines indicate 95% confidence limits, the range within which 95% of results typically fall. Erythroid cell yields were significantly higher in SFEM II compared to ACF ($p < 0.01$). Erythroid cell yields in SFEM II were also significantly higher than in SFEM for 5 of 6 CB samples tested ($p < 0.05$).

Applications:

- Research into the regulation of erythropoiesis
- Development of procedures to expand erythroid cells in culture
- Assessment of efficacy and toxicity of candidate therapeutics on erythroid progenitor cells for drug development


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 hematopoietic stem and progenitor cells.
StemSpan™ SFEM II 09605 (100 mL) 09655 (500 mL)	Enhanced version of StemSpan™ SFEM.	Serum-free expansion of human hematopoietic stem and progenitor cells. Generation of mature blood cells by differentiation of lineage-specific progenitor cells.
StemSpan™-ACF 09805 (100 mL) 09855 (500 mL)	Animal component-free (ACF) medium containing only recombinant and synthetic components.	Culture of human hematopoietic stem and progenitor cells in the absence of animal- or human-derived components.
StemSpan™ Erythroid Expansion Supplement (100x)  02692 (1 mL)	Pre-mixed cocktail of recombinant human cytokines (SCF, IL-3, EPO).	Generation of erythroid cells by expansion and lineage-specific differentiation of human hematopoietic progenitor cells.

Table 2. StemSpan™ serum-free expansion media and Erythroid Expansion Supplement.

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