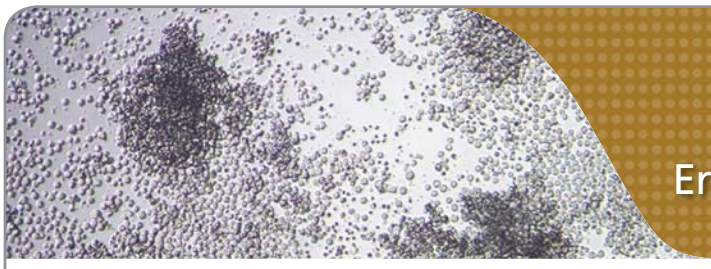

StemSpan™

Erythroid 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 (HSPCs) isolated from human bone marrow (BM), cord blood (CB) or other tissues. STEMCELL Technologies has developed a family of expansion media, which includes serum-free and animal component-free (ACF) 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™ 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 PB. It is optimized for use in combination with StemSpan™ serum-free SFEM (Catalog #9600/09650), SFEM II (Catalog #09605/09655) and ACF Erythroid Expansion (ACF-E) media (Catalog #09955).

StemSpan™ ACF-E is a new animal component-free (ACF) medium that has been specifically developed for the expansion and erythroid differentiation of human CD34$^+$ cells, when combined with the StemSpan™ Erythroid Expansion Supplement. StemSpan™ ACF-E is recommended for applications where the use of a medium devoid of animal- and human-derived components is required.

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™ SFEM, SFEM II and ACF-E

Representative Results

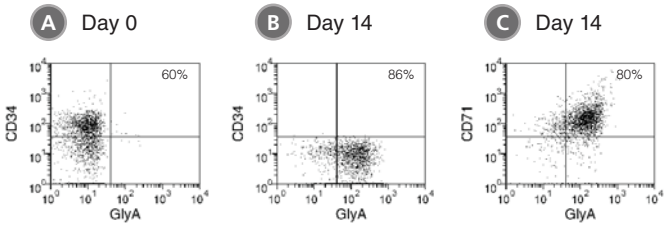

Figure 1. CD71$^+$GlyA$^+$ Cells Produced from CB-Derived CD34$^+$ Cells After 14 Days of Culture in StemSpan™ SFEM Containing StemSpan™ Erythroid Expansion Supplement

CB-derived CD34$^+$ cells were cultured in StemSpan™ SFEM containing Erythroid Expansion Supplement. Cells were stained and analyzed for HSPC marker CD34 and erythroid markers CD71 and GlyA (A) before culture and (B,C) after 14 days of culture. 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 the cells after 14 days of 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.

Table 1. Production of Erythroid Cells from Human CB-Derived CD34$^+$ Cells Cultured in StemSpan™ Media Containing StemSpan™ Erythroid Expansion Supplement

MEDIUM	ERYTHROID CELLS PRODUCED PER INPUT CD34$^+$ CELL (95% CL*)	% ERYTHROID CELLS (95% CL*)
SFEM	98300 (58400 - 138200)	99 (98 - 100)
SFEM II	298800 (205500 - 392000)	99 (99 - 100)
ACF-E	246900 (172600 - 321200)	97 (95 - 99)

Yield and percentages of erythroid cells produced after culturing purified CD34$^+$ CB cells (n = 15) for 14 days in StemSpan™ SFEM, SFEM II or ACF-E media containing Erythroid Expansion Supplement. Erythroid cells were identified by flow cytometry after staining with antibodies against CD71 and GlyA. The % erythroid cells represent the percentage of cells that express CD71 and/or GlyA.
*CL: confidence limits.

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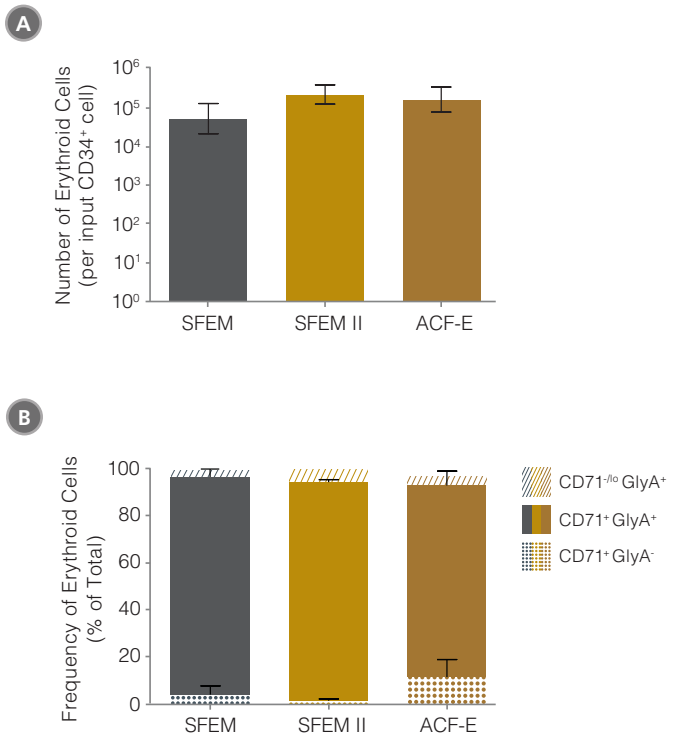

Figure 2. Production of Erythroid Cells from Human CB-Derived CD34⁺ Cells Cultured in StemSpan™ Media Containing StemSpan™ Erythroid Expansion Supplement

(A) Average numbers of erythroid cells generated after culturing purified CD34⁺ CB cells (n=15) for 14 days in StemSpan™ SFEM (gray bars), SFEM II (gold bars) or ACF-E (brown bars) media containing Erythroid Expansion Supplement. Shown are the number of erythroid cells that express CD71 and/or GlyA per input CD34⁺ cell. (B) The percentages of the different erythroid cell subsets generated in these cultures are shown, including $CD71^{-/lo}GlyA^{+}$ normoblasts, $CD71^{+}GlyA^{+}$ erythroblasts, and immature $CD71^{+}GlyA^{-}$ erythroid progenitors and pro-erythroblasts. Vertical lines indicate 95% confidence limits (CL). Erythroid cell yields were significantly higher in SFEM II and ACF-E than in SFEM ($p < 0.05$; paired t-test, n = 15). See Table 1 for values and 95% CL.

Applications:

- Research into the regulation of erythropoiesis
- Development of procedures to expand erythroid cells in culture
- Assessment of effects of candidate therapeutics on erythroid progenitor cells during drug development
- Generation of somatic target cells for reprogramming into induced pluripotent stem (iPS) cells

Media and Supplements for Erythroid Cell 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™ Erythroid Expansion Medium (ACF) 09955 (500 mL)	Animal component-free (ACF) medium containing only recombinant and synthetic components	Expansion and differentiation of human CD34⁺ cells into erythroid progenitor cells in the absence of non-human animal-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 CD34⁺ 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**.