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## Product Information

### **Stemline™ Hematopoietic Stem Cell Expansion Medium** without antibiotics or cytokines

Product Code **S 0189**

### **Stemline™ II Hematopoietic Stem Cell Expansion Medium** without antibiotics or cytokines

Product Code **S 0192**

Storage Temperature 2-8 °C

Synonyms: Hematopoietic Progenitor Cell Expansion Medium, CD34<sup>+</sup> Expansion Medium

#### **Product Description**

The use of human Hematopoietic Stem Cells (HSC) isolated from umbilical cord blood (CB) as a source of cellular reconstitution following high-dose chemotherapy is now a common therapeutic modality for the treatment of malignancy. However, due to the low yield of HSC from CB, HSC transfusion is most effective in children and has limited application in adults. In order to obtain sufficient numbers of cells for applications of this therapeutic approach in adults, *ex vivo* expansion has been utilized to ensure successful engraftment and minimize the short-term effects of neutropenia and thrombocytopenia.

We have developed two serum-free media formulations for the optimal expansion of CB HSC, Stemline™ Hematopoietic Stem Cell Expansion Medium (Product No. S 0189) and Stemline™ II Hematopoietic Stem Cell Expansion Medium (Product No. S 0192). These media promote the expansion of CD34<sup>+</sup> hematopoietic stem cells derived from umbilical cord blood and are also effective for the *ex vivo* expansion of HSC isolated from bone marrow or mobilized peripheral blood. These media support high viable cell densities. The elimination of serum reduces variability in the performance of the media and eliminates safety risks associated with possible adventitious agents in serum.

#### **Intended Use**

These products are intended for research use only. The safety and efficacy of these products have not been established for clinical applications.

#### **Components**

Stemline™ Hematopoietic Stem Cell Expansion Medium (Product No. S 0189) and Stemline™ II Hematopoietic Stem Cell Expansion Medium (Product No. S 0192) are both proprietary formulations without antibiotics and cytokines. The Stemline Hematopoietic Stem Cell Expansion Medium (Product No. S 0189) formulation also does not contain glutamine.

Human serum albumin is the only animal-derived protein in both media. It has been found to be non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV and HB<sub>s</sub>Ag. Handle as if potentially infectious. Stemline™ II Hematopoietic Stem Cell Expansion Medium (Product No. S 0192) contains no other animal-derived components.

#### **Preparation Instructions**

Media are supplied as sterile liquids (1X) and must be supplemented with cytokines and antibiotics, if desired. Stemline™ Hematopoietic Stem Cell Expansion Medium (Product No. S 0189) must also be supplemented with glutamine. Add 20 ml of 200 mM L-glutamine solution or 0.5844 g powder (irradiated) per liter of medium.

#### **Storage/Stability**

These media are stable, when stored at 2-8 °C and protected from light, until the date indicated on the label.

## Procedure

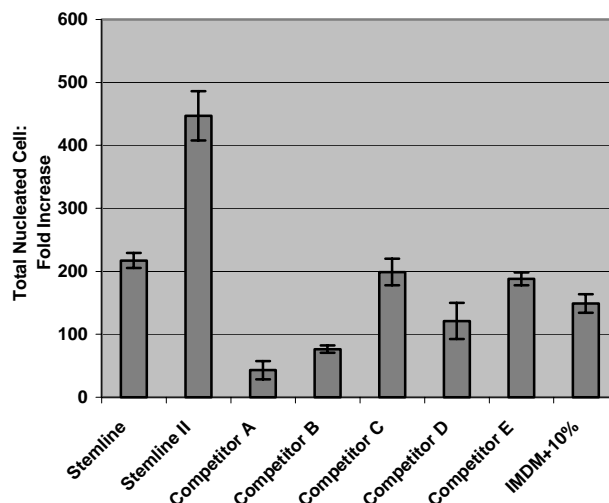
### Plating Cultures

1. Prepare either fresh or frozen CD34<sup>+</sup> cells as directed by the supplier or in accordance with established protocols.
2. Count cells using a hemacytometer.
3. Transfer the proper number of cells to the desired culture vessel with medium supplemented with cytokines (and antibiotics if desired).
4. Place the culture vessel in a humidified incubator at 37 °C and 5% CO<sub>2</sub>.

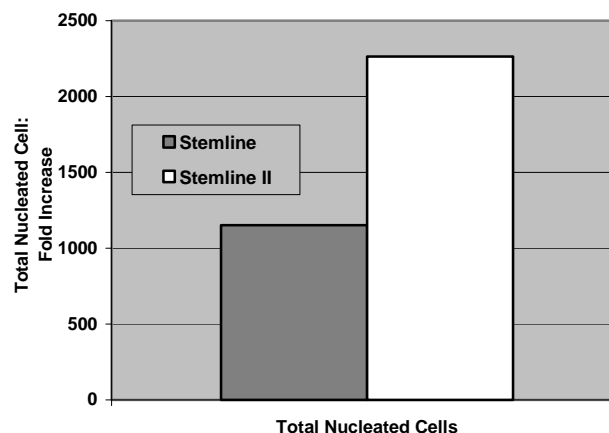
### Product Profile

Sigma's Stemline™ Hematopoietic Stem Cell Expansion Media (Product No. S 0189 and S 0192) demonstrate excellent expansion of total nucleated cells (TNC), committed progenitors (CD34<sup>+</sup>/CD38<sup>+</sup>) and primitive progenitors (CD34<sup>+</sup>/CD38<sup>-</sup>). These products were compared with several other commercially available serum-free expansion media for their ability to expand cord blood CD34<sup>+</sup> cells in a 24-well microplate culture system. For these small-scale experiments, triplicate 1-ml cultures of 10,000 cord blood CD34<sup>+</sup> cells/ml were incubated for 10 days in Stemline™ media or other commercial products containing 100 ng/ml each of SCF (Stem Cell Factor, Product No. S 7901), G-CSF (Granulocyte-Colony Stimulating Factor, Product No. G 0407) and TPO (Thrombopoietin, Product No. T 1568).

The study was then expanded to a 2-step, clinical-scale protocol using Teflon® culture bags, which were assayed for TNC expansion prior to transplantation into NOD/SCID mice. For clinical-scale studies, cord blood CD34<sup>+</sup> cells were cultured for 7 days in 100 ml Teflon® culture bags containing 50 ml of culture medium plus cytokines. Cells were harvested from these bags and a 5-ml aliquot was transferred to a second 100 ml Teflon® bag containing 50 ml of selected medium plus cytokines and cultured for an additional 7-day culture period. At the end of both culture protocols, cells were harvested and assayed as previously described. The data are summarized in Figures 1, 2, and 3. Both media demonstrated superior expansion of total nucleated cells (TNC), as well as both committed and primitive progenitors. The expanded cells were able to successfully engraft (first and second recipient) NOD/SCID mice (data not shown).

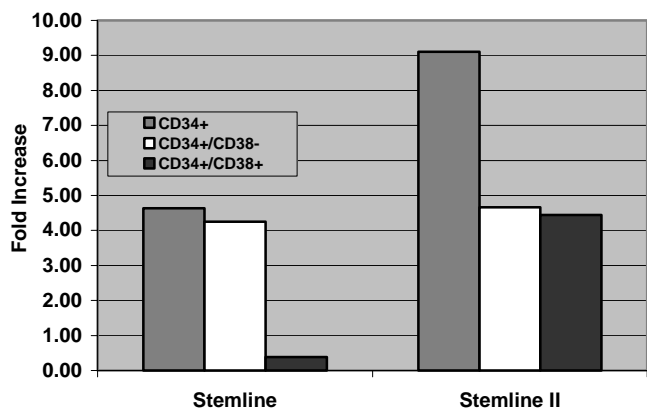


**Figure 1. Comparison of Stemline Media to Commercially Available Products.** Cells were seeded in triplicate at 10,000 cells per well in 24-well tissue culture plates containing either Stemline™ Hematopoietic Stem Cell Expansion Medium (Product No. S 0189), Stemline™ II Hematopoietic Stem Cell Expansion Medium (Product No. S 0192) or one of several leading competitors' media. The wells were supplemented with 100 ng/ml each of SCF, G-CSF, and TPO. Each well was trituated and harvested after a 10-day expansion. TNC were counted using a hemacytometer and averaged for all 3 wells in each condition.



**Figure 2. Comparison of Stemline Media in Clinical-Scale 14-Day Expansions in Teflon® Bags.** Cells were seeded in Teflon® culture bags containing either Stemline™ Hematopoietic Stem Cell Expansion Medium (Product No. S 0189) or Stemline™ II Hematopoietic

Stem Cell Expansion Medium (Product No. S 0192). The bags were supplemented with 50 ng/ml of FLT3L and 100 ng/ml each of SCF, G-CSF, and TPO. The cells were expanded for 7 days prior to being harvested and seeded into a new culture bag for an additional 7 days. Cells were harvested from the second culture bag and TNC were counted. These expanded cells were used to successfully engraft NOD/SCID mice (data not shown).



**Figure 3. Flow Cytometric Analysis of CD34<sup>+</sup> Cord Blood Cells from the Clinical-Scale Expansion in Teflon<sup>®</sup> Bags.** Cells were expanded as previously described and assayed for CD34 and CD38 using standard flow cytometry procedures.

#### References

1. Weissman, I.L., Stem Cells: Units of Development, Units of Regeneration, and Units of Evolution. *Cell*, **100**, 157-168 (2000).
2. McAdams, T.A., Miller, W.M., Papoutsakis, E.T., Hematopoietic Cell Culture Therapies (Part I): Cell Culture Considerations. *Trends in Biotechnology*, **14**, 341-349 (1996).
3. Möbest, D., Mertelsmann, R., Henschler, R., Serum-Free ex Vivo Expansion of CD34<sup>+</sup> Hematopoietic Progenitor Cells. *Biotechnology and Bioengineering*, **60(3)**, 341-347 (1998).
4. Allison, D.W., Leugers, S.L., Pronold, B.J., Van Zant, G., Donahue, L.M., Improved Ex Vivo Expansion of Functional CD34<sup>+</sup> Cells Using Stemline II Hematopoietic Stem Cell Expansion Medium. Presented at the American Society for Hematology Annual Meeting, (2003).

#### Precautions and Disclaimer

MSDS is available upon request or at [www.sigma-aldrich.com](http://www.sigma-aldrich.com). Teflon is a registered trademark of E.I. du Pont de Nemours & Co., Inc.

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