3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Stemline® XF MSC Basal Medium
Catalog Number **14371C**
Storage Temperature 2–8 °C

Stemline® XF MSC Supplement
Catalog Number **14372C**
Storage Temperature –20 °C

Stemline® XF MSC Medium Kit
Catalog Number **14373C**, Contains Basal Medium (14371C-500ML) and Supplement (14372C-5ML)
Refer to kit components for recommended storage information. Available in US Only

TECHNICAL BULLETIN

Product Description

The use of mesenchymal stromal/stem cells (MSCs) isolated from multiple sources as a therapeutic agent in cell therapies has been a target of clinical studies for some time. However, the limitations of procurable cells from specific tissues and doubling capacity of primary cells determine the necessity of *ex vivo* expansion. Typical static *in vitro* culture methods are often too cumbersome and inefficient to support commercial scale production of MSCs. Single-use stirred tank bioreactor systems are a platform that can address this limitation and have been proven effective for microcarrier-based production of adherent cell therapies. Cell culture medium and supplements are critical factors of the scale-up process. Many processes currently include fetal bovine serum (FBS) which is a commonly-used supplement associated with regulatory, supply, and consistency challenges.

Stemline XF MSC Basal Medium and Supplement promote the expansion of MSCs of human origin with high viable cell densities in multiple platforms without the use of FBS. This culture medium exhibits consistent growth performance in both planar and suspension-based systems, and hMSCs expanded in this medium maintain all the characteristics of multipotency and immunomodulatory activity. All Stemline XF MSC products are manufactured using raw materials selected for high quality and suitability for manufacturing applications and do not contain phenol red. The elimination of serum reduces variability in the performance of the medium and eliminates safety risks associated with possible adventitious agents in serum.

The Stemline XF MSC medium is a proprietary formulation. This medium does not contain L-glutamine, antibiotics, or fetal bovine serum.

Components

The medium and supplement (Catalog Numbers 14371C and 14372C) may be purchased individually, or together as the Stemline XF MSC Medium Kit (Catalog Number 14373C), which includes 500 mL of 14371C and 5 mL of 14372C. Kit available in US Only

Precautions and Disclaimer

For research or further manufacturing use only. Not for direct use in humans or animals. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

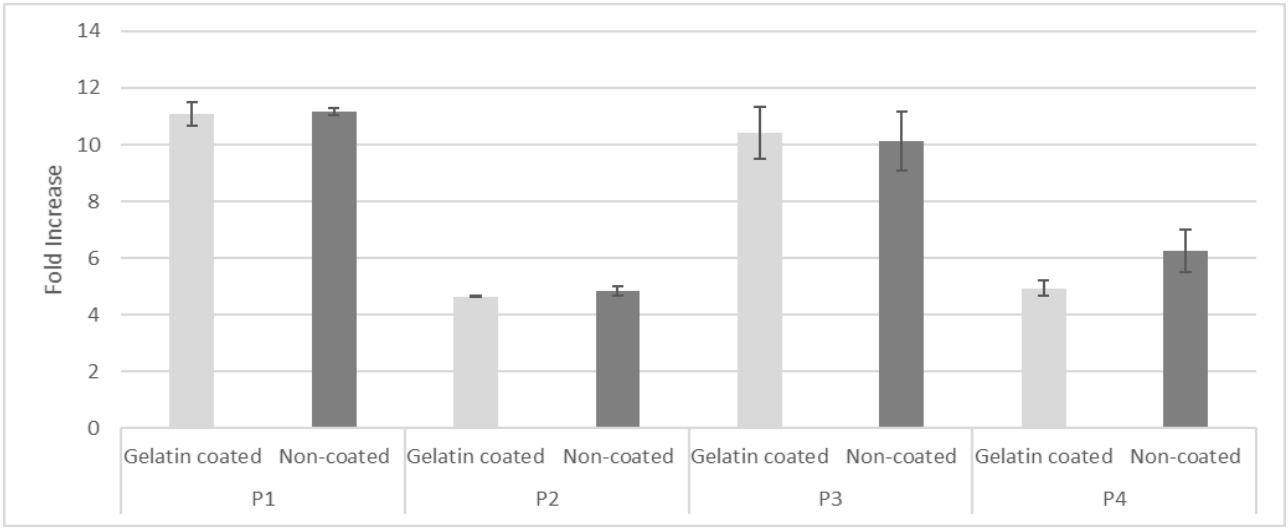
The Basal Medium and Supplement are supplied as sterile-filtered liquids (1× and 100×, respectively). The Stemline XF MSC Supplement (Catalog Number 14372C) should be thawed and used immediately by adding the full volume of supplement to the full volume of Stemline XF MSC Medium (Catalog Number 14371C) in a ratio of 1:100, avoiding additional freeze-thaw cycles. This medium does not contain L-glutamine and should be supplemented as necessary based on the MSC cell line requirements.

Storage/Stability

The medium is stable when stored appropriately and protected from light, until the date indicated on the label.
The supplement is stable when stored appropriately and protected from light.

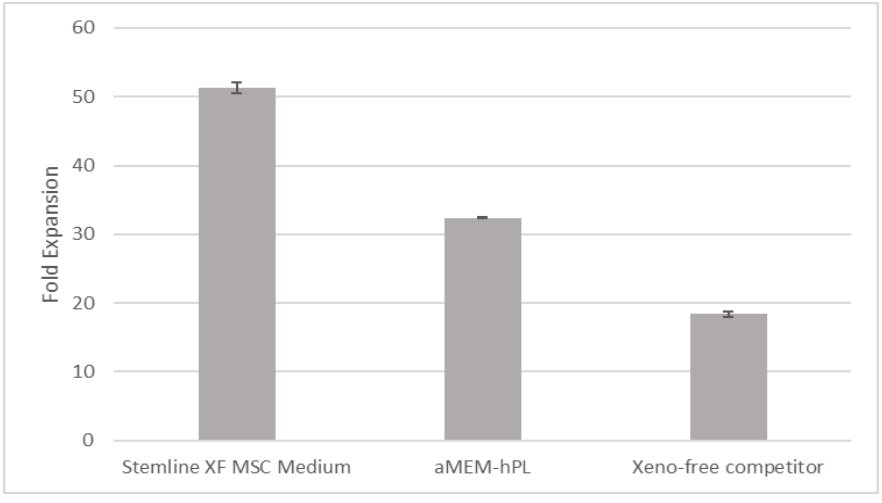
Results
The supplemented medium (Catalog Numbers 14371C and 14372C) shows robust expansion of mesenchymal stem cells. It was compared with aMEM-hPL and commercially available xeno-free (XF) competitors. Cells expanded in Stemline XF MSC medium retain their differentiative potential, immunomodulatory activity, and are able to be passaged routinely.

Figure 1.
Surface Coating



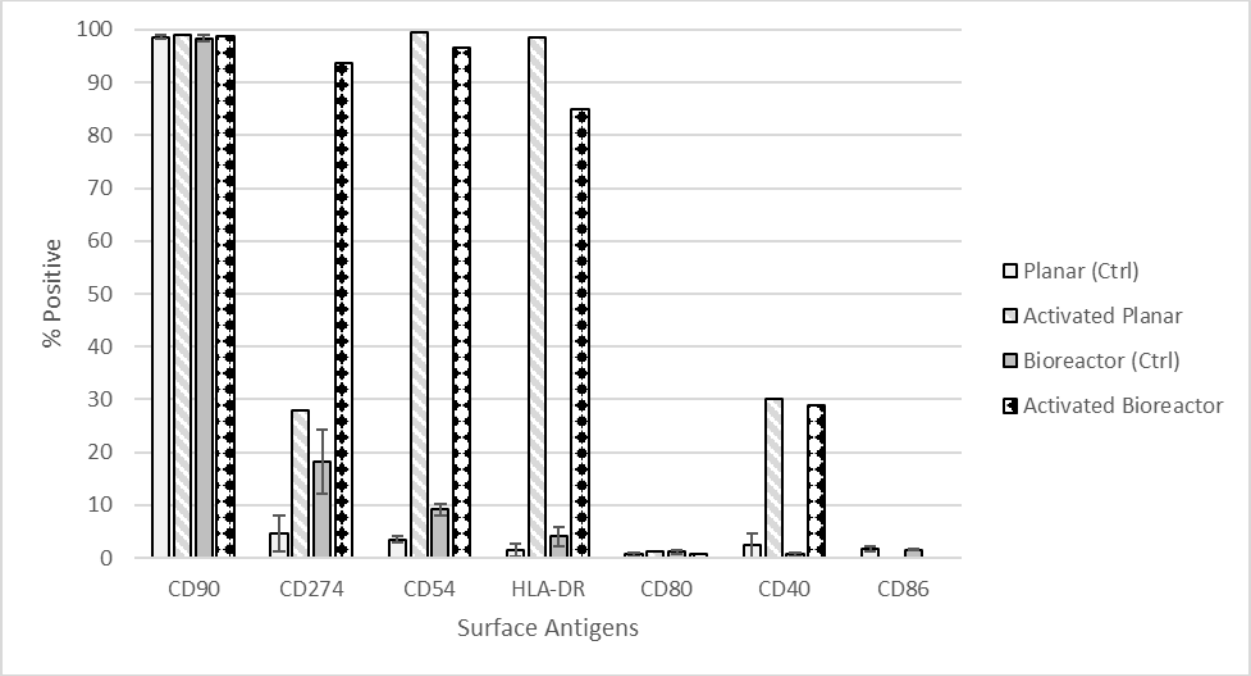
Cells were seeded in triplicate at 3,000 cells per cm^2 in gelatin-coated 6-well plates or tissue culture treated 6-well plates containing Stemline XF MSC Medium. A commercially available vial of bone marrow derived MSCs was thawed and cultured under these conditions for a total of four passages. Cells achieved similar fold increases regardless of surface coating.

Figure 2.
Media Comparison



Cells were seeded in duplicate at 3,000 cells per cm^2 in a Mobius® 3L Stirred Tank Bioreactor containing either Stemline XF MSC Medium or the media indicated. Cells were seeded onto collagen-coated microcarriers and cultured for 8 days with the same feeding schedule. MSCs were counted using NC-200 and average viable cell count was determined for each condition.

Figure 3.
Levels of Surface Antigens



Cells expanded in Stemline XF MSC medium in planar or suspension-based formats retain characteristic ability to modulate presentation levels of immune-related surface antigens. "Activated" conditions were exposed to INFγ, while control conditions were not.

References

1. Galipeau, J., *et al.* International Society for Cellular Therapy perspective on immune functional assays for mesenchymal stromal cells as potency release criterion for advanced phase clinical trials. *Cytotherapy*, Feb: **18(2)**, 151-159 (2016).
2. Le Blanc, K., Immunomodulatory effects of fetal and adult mesenchymal stem cells, *Cytotherapy*, **5(6)**, 485-489 (2003). https://doi.org/10.1080/14653240310003611.
3. Dominici, M. *et al.,* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement, *Cytotherapy*, **8(4)**, 315-317 (2006). DOI: 10.1080/14653240600855905
4. Schnitzler, A.C., et al., Bioprocessing of human mesenchymal stem/stromal cells for therapeutic use: Current technologies and challenges, *Biochemical Engineering Journal*, **108**, 3-13 (2016). https://doi.org/10.1016/j.bej.2015.08.014.

Troubleshooting Guide

1. **What should I expect when transitioning from serum-containing medium to xeno-free medium?**

   Transitioning MSCs from serum containing medium to Stemline XF MSC medium does not require an adaptation protocol. More generally, some hMSCs have difficulty with attachment in a xeno-free medium. We have achieved good results with both gelatin-coated and non-coated surfaces (see Figure 1). If the cell line of interest is having difficulty with attachment, a coating solution may be beneficial.

2. **What is the recommended concentration of L-Glutamine for hMSC culture?**

   We recommend adding 200 mM L-Glutamine solution (Catalog Number 59202C) to a final concentration of 2 mM.
   Note: L-Glutamine consumption in microcarrier-suspension format may be increased for some cell lines, so that concentration could be adjusted or taken into consideration when determining a feed strategy.

3. **Are any additives required for microcarrier-based suspension cultures?**

   When culturing in microcarrier-based suspension platforms, we supplement with an anti-shear protectant and an antifoaming agent. While it is not necessary, we also found use of antibiotics did not significantly affect cell growth in our studies. However, some studies indicate that cells grown without serum may be more sensitive to antibiotic usage.