

## Product Information

### Stemline™ Dendritic Cell Maturation Medium without L-glutamine

Product Code **S3444**

Storage Temperature 2-8°C

Synonyms: D Cell Maturation Medium

#### Product Description

Stemline™ Dendritic Cell Maturation Medium has been developed to support the optimal maturation of Dendritic Cells from human CD14<sup>+</sup> monocytes. This medium supports high viable cell densities. The elimination of serum reduces performance variability in the medium and eliminates safety risks associated with possible adventitious agents in serum.

#### Intended Use

*For R&D use only. Not for drug, household, or other uses.*

#### Introduction

Dendritic Cells (DC) are considered to be the most potent of antigen presenting cells. Consequently, they play a pivotal role in the generation of primary T-Cell responses through their ability to process and present antigens, while delivering co-stimulatory signals and secreting cytokines necessary for T-Cell triggering.<sup>1-3</sup> Traditionally, cell culture media have been supplemented with serum to support the *in vitro* growth of DCs. However, specific cytokines in serum, such as TGF-β, and many other undefined components may affect the differentiative potential of DC precursors and the functional capacity of mature DCs. The ability of these DCs to efficiently present antigens and thus play a central role in the orchestration of the adoptive immune responses have made these cells a recent focus of interest in the conception of vaccine strategies. Few advances have been made in engineering a DC product specifically optimized for this purpose. At present, there are several serum-free formulations available which exhibit a limited potential for the generation of mature DCs. To this end, we have developed a serum-free medium exclusively formulated to generate large numbers of mature DCs that have been characterized morphologically, phenotypically, and functionally.

Stemline Dendritic Cell Maturation Medium is a serum-free formulation optimized specifically for the culture of human DCs from CD14<sup>+</sup> monocytes.

Stemline Dendritic Cell Maturation Medium outperforms other commercially available serum-free media in the generation of mature DCs.

#### Components

Stemline Dendritic Cell Maturation Medium is a proprietary formulation. The medium does not contain L-glutamine, antibiotics or cytokines. Human serum albumin and human transferrin are the only human origin materials and are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV and HB<sub>s</sub>Ag. Handle as if potentially infectious.

#### Preparation Instructions

This medium is supplied as a sterile 1X liquid. **Stemline Dendritic Cell Maturation Medium must be supplemented with L-glutamine (2 mM final concentration), desired cytokines and/or antibiotics.**

#### Storage/Stability

This medium is stable, when stored at 2-8 °C and protected from light, until the date indicated on the label.

#### Procedure

##### Plating Cultures

1. Prepare fresh CD14<sup>+</sup> or equivalent monocytes in accordance with established protocols.
2. Count cells using a hemacytometer.
3. Transfer the proper number of cells to the desired culture vessel, containing medium supplemented with GM-CSF and IL-4 (and antibiotics if desired).
4. Place the culture vessel in a humidified incubator at 37°C and 5% CO<sub>2</sub> for 6 days.
5. Mature cells with TNF-α and PGE<sub>2</sub> for an additional 2 days in a humidified incubator at 37°C and 5% CO<sub>2</sub>

#### Product Profile

Sigma's Stemline™ Dendritic Cell Maturation Medium (Product# S3444) shows rigorous maturation of Dendritic Cells from CD14<sup>+</sup> monocytes. This product was compared with several other commercially available serum-free expansion media for their ability to support the maturation of DCs in a multi-well microplate culture system.

For these small-scale experiments, duplicate cultures at 1,000,000 CD14<sup>+</sup> monocytes/ml were incubated for 6 days in Stemline medium or other commercial product containing GM-CSF and IL-4. To obtain mature DCs, the cells were treated for another 48 hours with TNF- $\alpha$  and PGE<sub>2</sub>.

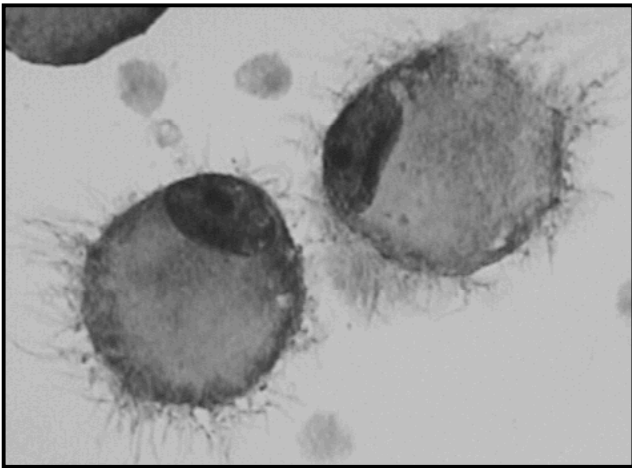
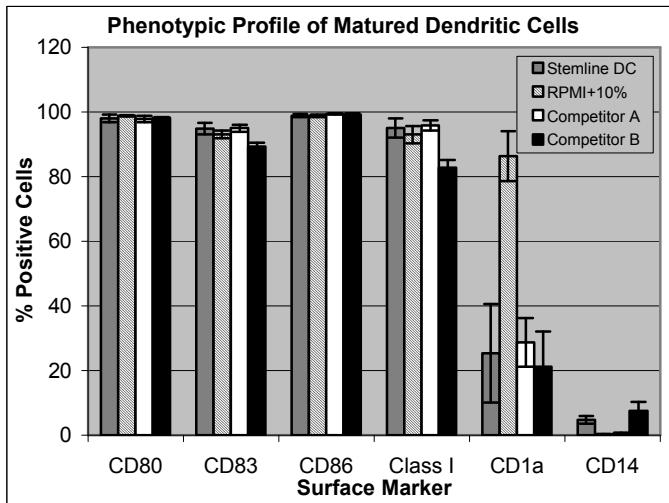
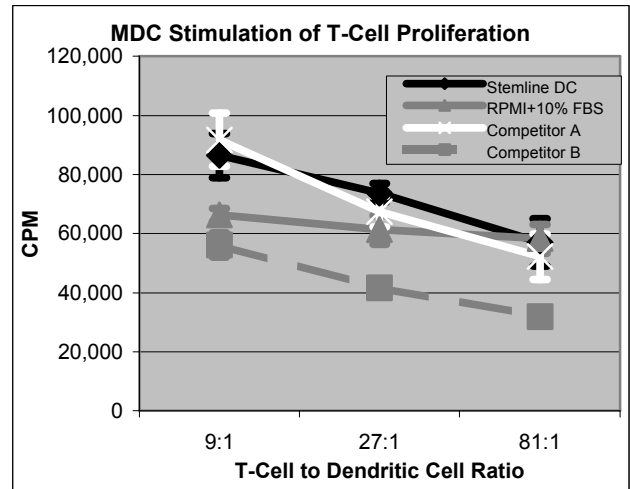


Figure 1. Dendritic Cells matured in Stemline Dendritic Cell Maturation Medium show typical morphological markers of maturity such as large veiled, dendritic processes.



Graph 1. Dendritic Cells matured in Stemline Dendritic Cell Maturation Medium show optimal levels of phenotypic markers of maturation such as, CD80, CD83, CD86, Class I, CD1a and CD14. Cells were seeded at 1,000,000 cells per well in tissue culture plates containing either Stemline Dendritic Cell Maturation Medium or 2 leading competitors. Each well was trituated and harvested after an 8-day maturation.



Graph 2. Proliferative T-Cell response in mixed lymphocyte culture. T-cells were stimulated by mature dendritic cells matured in either Stemline Dendritic Cell Maturation Medium or the other commercially available media. An indicator of mature dendritic cell function, increasing the ratio of T-cells to dendritic cells (stimulator to effector ratio) still results in high levels of T-cell proliferation.

### References

1. Banchereau, J. and Palucka, A. K., Dendritic Cells as Therapeutic Vaccines Against Cancer. *Nat Rev Immunol*, **5(4)**, 296-306 (2005)
2. Lanzavecchia, A. and Sallusto, F., Regulation of T Cell Immunity by Dendritic Cells. *Cell*, **106(3)**, 263-266 (2001)
3. Mellman, I. and Steinman, R. M., Dendritic Cells: Specialized and Regulated Antigen Processing Machines. *Cell*, **106(3)**, 255-258 (2001)

### Precautions and Disclaimer

MSDS is available upon request or at [www.sigma-aldrich.com](http://www.sigma-aldrich.com).

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