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™ T-Cell Expansion Medium
Without L-glutamine

Product Code **S1694**
Storage Temperature 2-8°C

Synonyms: T-Cell Growth Medium

Product Description

Stemline™ T-Cell Expansion Medium has been developed to promote the optimal expansion of T-Cells of human origin. 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

The immune system is composed of many different types of white blood cells the most common of which is the lymphocyte. A specialized type of lymphocyte called a T-cell is responsible for orchestrating the cellular arm of the immune response against cancer or infectious diseases[1]. There are a variety of medical conditions in which patients' T-cells are present in low numbers or are not functioning properly. These clinical conditions place patients at high risk for infections and cancer. Adoptive immunotherapy is the *ex-vivo* manipulation and expansion of antigen specific T-Cells for subsequent administration into patients[2]. The effectiveness of T-cell mediated immunotherapy depends upon a number of conditions after *ex vivo* expansion such as the fold expansion, the functionality, polyclonality, and antigen-specificity of the T-cells[3]. To this end, we have developed a serum-free medium, Stemline T-Cell Expansion Medium for the optimal expansion of T-cells.

Components

Stemline T-Cell Expansion Medium is a proprietary formulation. The medium does not contain 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_sAg. Handle as if potentially infectious.

Preparation Instructions

This medium is supplied as a sterile (1X) liquid and must be supplemented with stimulatory antibodies, cytokines and/or antibiotics, if desired. Stemline™ T-Cell Expansion Medium must also be supplemented with L-glutamine. Add 20 ml of 200mM L-glutamine solution or 0.584 g powder (irradiated) per liter of medium.

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

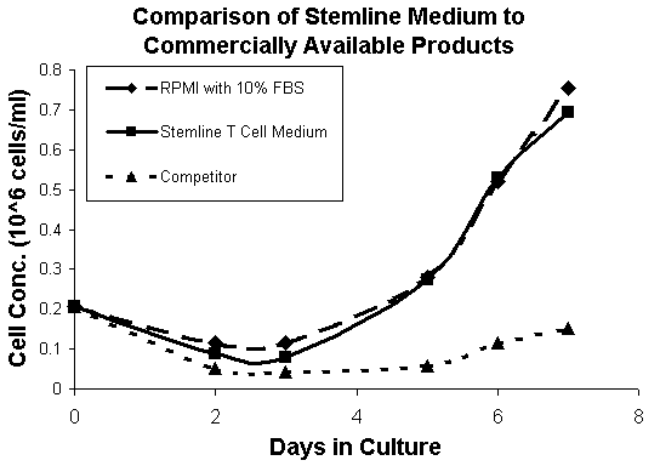
1. Prepare either fresh or frozen PBMNCs (peripheral blood mononuclear cells) as directed by the supplier or in accordance with established protocols.
2. Count cells using a hemacytometer.
3. Transfer the proper number of cells to the desired culture vessel containing medium supplemented with cytokines and stimulatory antibodies (and antibiotics if desired).
4. Place the culture vessel in a humidified incubator at 37°C and 5% CO_2.

Product Profile

Sigma's Stemline T-Cell Expansion Medium (Product number S1694) demonstrated rigorous expansion of T-cells from PBMNCs. This product was compared with several other commercially available serum-free expansion media for their ability to expand T-cells in T75 culture flasks. For these small-scale experiments, 200,000 PBMNCs/ml were incubated for up to 7 days in Stemline medium or other commercial product containing 100 IU/ml IL-2 and anti-CD3 (OKT3, 20 ng/ml) antibody.

The expanded T-Cell population from these experiments was then used in ^{51}Chromium release assays to test for functional cytolytic potential. Briefly, target cells (K562, a human chronic myelogenous leukemia cell line), were labeled with ^{51}Chromium and linked to anti-CD3 (OKT3) via F_c receptor. When mixed with effector or cytolytic T-cells, the target cells undergo apoptosis or lysis and release ^{51}Chromium. The amount of ^{51}Chromium released into the supernatant is proportional to the number of targets killed and the number of functional cytolytic T-Cells.

Figure 1. Peripheral Blood mononuclear cells were incubated in either RPMI 1640 + 10% FBS, Competitor Medium or Stemline T-Cell Expansion Medium. Cells were stimulated using OKT3 anti-CD3 (20ng/ml) in the presence of 100 U/ml IL-2.



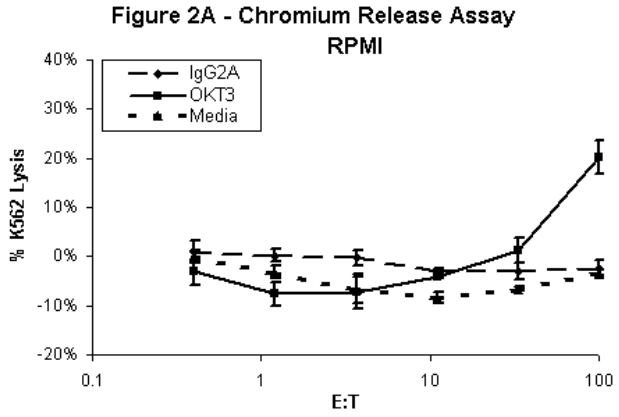
References

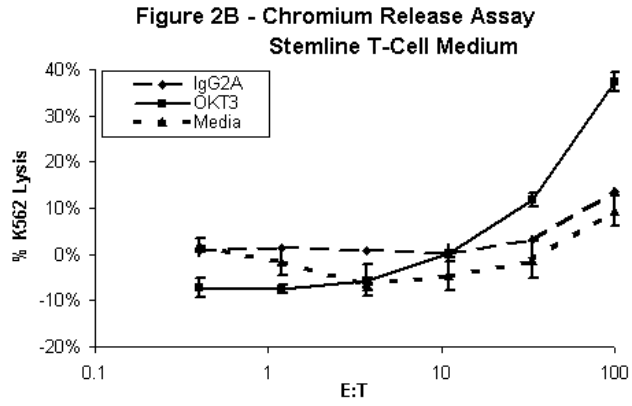
1. Williams, D. A. in Hematology: *Basic Principles and Practice* (2000), Hoffman et al (eds.), Churchill Livingstone, 126-138
2. Dudley, M.E. and Rosenberg, S.A., Adoptive-cell-transfer therapy for the treatment of patients with cancer. Nat Rev Cancer, **3(9)**, 666-675 (2003)
3. Knutson, K.L., Almand, B., Mankoff, D.A., Schiffman, K., and Disis, M.L. Adoptive T-Cell Therapy for the treatment of solid tumors. Expert Opin. Biol. Ther. **2**(1), 1-12 (2002)

Precautions and Disclaimer

MSDS is available upon request or at www.sigma-aldrich.com.

Figure 2A/B. In order to measure cytolytic potential from the harvested T-cells in Figure 1, a re-targeted lytic assay was used. Briefly, anti-OKT3 was linked to chromium-labeled K562 target cells via F_c receptor. Harvested T-cells from either control medium (Fig. 2A, RPMI 1640 or Fig 2B, Stemline T-Cell Expansion Medium) were then incubated at various effector-to-target ratios and the level of polyclonal cytolytic activity was determined as percent K562 lysis.





Stemline is a trademark of Sigma-Aldrich® Biotechnology LP and Sigma-Aldrich Co.

DKF 11/04
Revised 06/05