

Serum-free T-Cell Expansion Medium

Product:

Product	Catalogue-No.	Size	Description
Serum-free T-Cell Expansion Medium	N103-1000	1000ml	without L-glutamine, used to promote the optimal expansion of T-Cells

Description:

Serum-free 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.

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. 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. 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. To this end, we have developed a serum-free medium, Serum-free T-Cell Expansion Medium for the optimal expansion of T-cells.

Storage/Stability

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

Composition:

Serum-free 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 HBsAg. Handle as if potentially infectious.

Preparation of complete media:

This medium is supplied as a sterile (1X) liquid and must be supplemented with stimulatory antibodies, cytokines and/or antibiotics, if desired. Serum-free 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.

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% CO₂.

Product Profile:

Nobimpex’s Serum-free T-Cell Expansion Medium (Product number N103-1000) 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 Serum-free medium or other commercial product containing 100 IU/ml IL-2 and anti-CD3 (OKT3, 20ng/ml) antibody.

The expanded T-Cell population from these experiment was then used in 51chromium 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 Fc receptor. When mixed with effector or cytolytic T-cells, the target cells undergo apoptosis or lysis and release 51Chromium. The amount of 51chromium release into the supernatant is proportional to the number of targets killed and the number of functional cytolytic T-Cells.

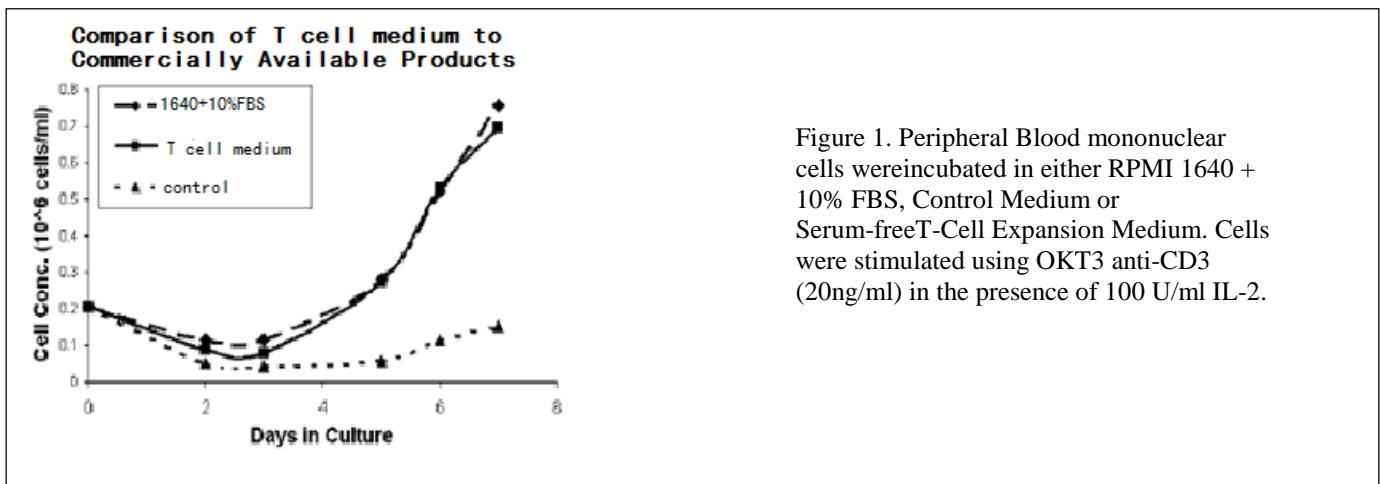


Figure 1. Peripheral Blood mononuclear cells were incubated in either RPMI 1640 + 10% FBS, Control Medium or Serum-free T-Cell Expansion Medium. Cells were stimulated using OKT3 anti-CD3 (20ng/ml) in the presence of 100 U/ml IL-2.

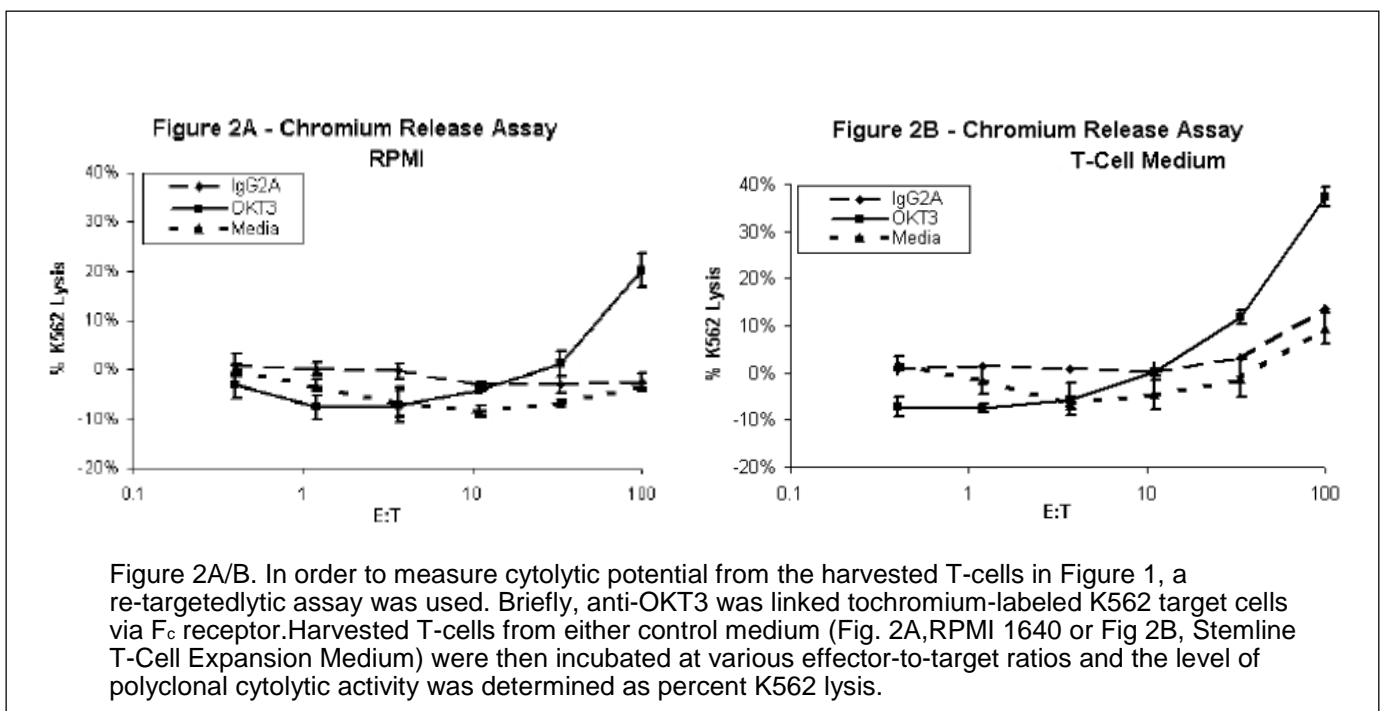


Figure 2A/B. In order to measure cytolytic potential from the harvested T-cells in Figure 1, a re-targeted lytic assay was used. Briefly, anti-OKT3 was linked to chromium-labeled K562 target cells via F_c receptor. Harvested T-cells from either control medium (Fig. 2A, RPMI 1640 or Fig 2B, Stemline T-Cell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