ExCellerate™ Human T Cell Expansion Media, Xeno-Free

Xeno-Free Media for Human T Cell Culture

Catalog Number: CCM030

Volume: 500 mL

PRODUCT DESCRIPTION

T Lymphocytes (T Cells) are a critical component of the adaptive immune response. While dysregulation of T cell function and proliferation contributes to the etiology of many diseases, the ability of T cells to activate an immune response toward specific antigens is being harnessed as a powerful immunotherapy tool to combat cancer and other diseases (1). To facilitate discovery and preclinical research for T cell immunoregulation and therapy, robust platforms for *ex vivo* expansion and maintenance of T cells, including specialized serum-free or xeno-free media, are required.

ExCellerate Human T Cell Expansion Media, Xeno-Free, is formulated and optimized for the *ex vivo* culture of human T lymphocytes in research applications. Unlike traditional serum-containing culture media, xeno-free media provides a stable culture and optimized environment, void of non-human animal-derived products, that facilitates the generation and expansion of T cells. The medium supports routine culture of human T cell lines or T cell clones and the stimulation of human peripheral blood lymphocytes. This product does not contain antibiotics.

INTENDED USE

ExCellerate Human T Cell Expansion Media, Xeno-Free, is a versatile media that can be used with a variety of cytokine and cell activation methods to facilitate the *ex vivo* culture of human T lymphocytes. The cell activation method and cytokine/growth factor combination used depends upon the experimental design.

STABILITY & STORAGE

Upon receipt, this media should be stored at 2-8 °C until the expiration date on the label.

PRECAUTIONS

The human origin-derived components used in this product have been derived from human plasma, which has been tested at the donor level and found to be negative for antibodies to HIV-1/2, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV). All other components are animal-free. However, the media should be handled as if potentially infectious. Safe laboratory procedures should be followed and protective clothing should be worn when handling this media. The acute and chronic effects of over-exposure to this media are unknown.

When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- This reagent should not be used beyond the expiration date indicated on the label.
- Results may vary due to variations among primary T lymphocyte populations derived from different donors.

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PROCEDURE FOR THE *EX VIVO* CULTURE OF HUMAN T CELLS

The protocol below describes the expansion of human T Cells using ExCellerate Human T Cell Expansion Media, Xeno-Free, in combination with the Cloudz™ T Cell Activation Kit (R&D Systems).

Note: *ExCellerate Human T Cell Expansion Media supports T cell expansion using bead-based or plate-bound anti-CD3/anti-CD28 antibodies along with optimized combinations of recombinant cytokines, including IL-2, IL-7, and IL-15. The activation and cytokine/growth factor combinations used with this media should be optimized by application or experimental protocol.*

OTHER MATERIALS REQUIRED

- Recombinant Human IL-2 (R&D Systems, Catalog # 202-IL)
- Cloudz™ T Cell Activation Kit (R&D Systems, www.rndsystems.com/cloudz)
- MagCellect™ Human $CD3^+$ T Cell Isolation Kit (R&D Systems, Catalog # MAGH101)
- Pipettes and pipette tips
- 24-well tissue culture plate
- 25 cm^2 tissue culture flask (T25)
- 75 cm^2 tissue culture flask (T75)
- Penicillin-Streptomycin
- 37 °C, 5% CO_2 incubator
- Inverted microscope
- Ficoll-Hypaque™
- Hemocytometer
- Centrifuge

REAGENT PREPARATION

ExCellerate Human T Cell Expansion Media – Determine the amount of media needed for your experiment. Warm the pre-determined volume of media to 37 °C.

ExCellerate Human T Cell Expansion Media can also be supplemented with cytokines and stored at 2-8 °C. This media will be stable for 2 weeks. Aliquots of cytokine-supplemented media can be warmed as to 37 °C as needed.

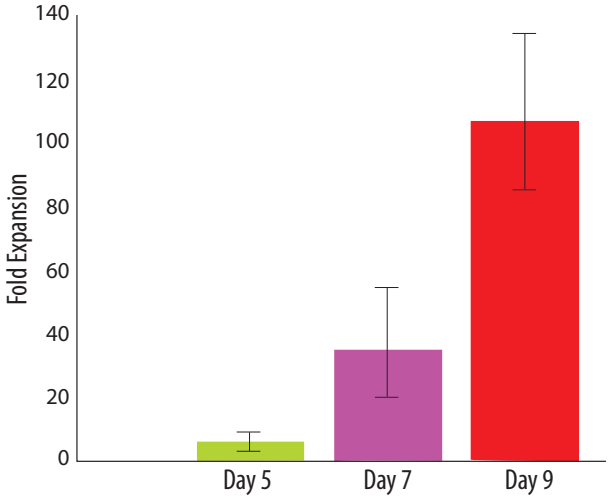
Note: *If needed, Penicillin-Streptomycin can be added to the ExCellerate Human T Cell Expansion Media at a 1:100 dilution.*

RECOMMENDED PROTOCOL

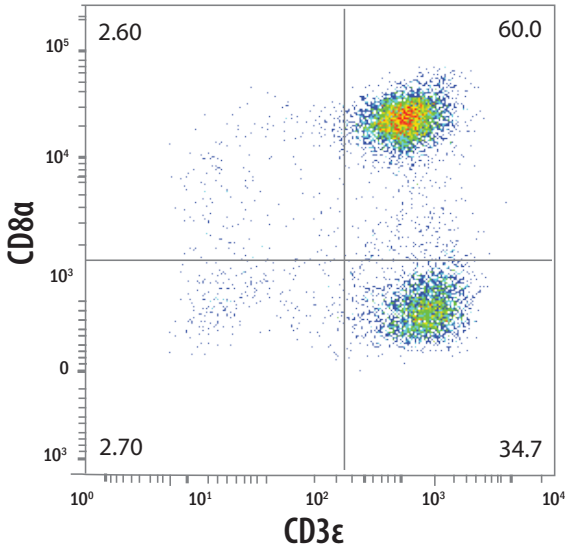
1. Prepare ExCellerate Human T Cell Expansion Media by adding Recombinant Human IL-2 (10-20 ng/mL).
   Note: *The optimal concentration of Recombinant Human IL-2 may vary depending on the experimental protocol (i.e. cell activation method, starting cell population).*
2. Isolate human peripheral blood mononuclear cells (PBMCs) from human blood using Ficoll-Hypaque density gradient centrifugation. **Optional:** If purified $CD3^+$ T cells are desired as a starting population, use the MagCellect™ Human $CD3^+$ T Cell Isolation Kit following the manufacturer's instructions.
3. Dilute cell suspension to 0.25 x 10^6 cells/mL in pre-warmed ExCellerate Human T Cell Expansion Media supplemented with Recombinant Human IL-2. In a 24-well plate, add 2 mL/well of the diluted cell suspension.
4. Following the Cloudz T Cell Activation Kit product insert instructions, add 25 µL/well of Cloudz CD3/CD28.
5. Incubate the cells at 37 °C and 5% CO_2 in a humidified incubator. This is Day 0.
6. On Day 2, transfer the cell suspension, including the Cloudz CD3/CD28, into a T25 flask. Add 2 mL of pre-warmed ExCellerate Human T Cell Expansion Media supplemented with Recombinant Human IL-2.
   Note: *Cells may stick to bottom of the well. Flush gently to ensure complete transfer.*
7. On Day 5, add 3 mL of pre-warmed ExCellerate Human T Cell Expansion Media supplemented with Recombinant Human IL-2.

8. On Day 7, transfer to a T75 flask, add 4 mL of pre-warmed ExCellerate Human T Cell Expansion Media supplemented with Recombinant Human IL-2.
9. Harvest the cells on Day 9 for desired downstream applications.
   a. If using cells for experimental applications, such as co-culture or adoptive transfer, Cloudz CD3/CD28 must be removed using Cloudz Release Buffer according to the product insert instructions for the Cloudz T Cell Activation Kit.
   b. If analyzing cells by flow cytometry, Cloudz CD3/CD28 microspheres can remain.

DATA EXAMPLES



Fold Expansion of Human T Cells using ExCellerate Human T Cell Expansion Media. Primary human peripheral blood mononuclear cells (PBMCs) were cultured for 9 days in ExCellerate T Cell Expansion Media, following the protocol provided in the product insert. Cell counts were performed to determine fold expansion compared to the Day 0 seeding density (0.25 x 10^6 cells/mL).



Phenotypic Analysis of Human T Cells Expanded in ExCellerate Human T Cell Expansion Media. Human PBMCs were cultured for 9 days in ExCellerate Human T Cell Expansion Media following the protocol provided in the product insert. Human T cells were enriched during the culture as indicated via the expression of positive T cell markers, including CD3 and CD8. The cells were labeled with a Mouse Anti-Human CD3 epsilon APC-conjugated Antibody (Catalog # FAB100A) and Mouse Anti-Human CD8 alpha PE-conjugated Antibody (Catalog # FAB1509).

REFERENCES

1. June, C.H. *et al*. (2018) Science **359**:1361.