ExCellerate™ Human T Cell Media Animal Component-Free

Catalog Number: CCM035

PRODUCT DESCRIPTION

ExCellerate Human T Cell Media, Animal Component-Free is formulated and optimized to support the robust expansion of T cells with a high percentage of cells expressing stem cell memory and central memory phenotype. ExCellerate Human T Cell Media is for the culture of human T lymphocytes.

INTENDED USE

ExCellerate Human T Cell Media is a versatile media compatible with various T cell activation methods, culture vessels (G-Rex and flasks), and different cytokine supplements for the culture of human T lymphocytes. The cytokine combination depends upon the experimental design of each researcher. This media supports the expansion of T cells in protocols using IL-7 and IL-15 cytokines alone or in various combinations.

STABILITY & STORAGE

Upon receipt, this media should be stored at 2-8 °C and **protected from light** until the expiration date on the Certificate of Analysis.

PRECAUTIONS

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.
- Results may vary due to variations among primary T cell lymphocyte populations derived from different donors.
- This reagent should not be used beyond the expiration date indicated on the label.

THE FOLLOWING MATERIALS ARE REQUIRED

- Recombinant Human IL-7 (R&D Systems®, # BT-007-AFL [Lyophilized] or BT-007-AFL/LQ [Liquid])
- Recombinant Human IL-15 (R&D Systems, # BT-015-AFL [Lyophilized] or BT-015-AFL/LQ [Liquid])
- Human CD3 Antibody
- Human CD28 Antibody
- 1X sterile PBS
- 24-well tissue culture plate
- 75 cm_2 tissue culture flask
- 37 °C, 5% CO_2 incubator
- Inverted microscope
- Hemocytometer
- Centrifuge
- G-Rex®6M Well Plate (Wilson Wolf™, # 80660M)
- Pipettes and pipette tips

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PROCEDURE FOR THE EX VIVO CULTURE OF HUMAN T CELLS

The protocols below describe the expansion of T cells using ExCellerate™ Human T Cell Media, Animal Component-Free.
Note: *The activation and cytokine combinations used with this media should be optimized by application or experimental protocol.*

REAGENT PREPARATION

Complete ExCellerate Human T Cell Media - Aliquot media and add 10 ng/mL rhIL-7 and 10 ng/mL rhIL-15. Media is stable for 2 weeks at 2-8 °C. Store protected from light.

RECOMMENDED PROTOCOL (G-Rex®6M Well Plate)

Day 0

1. Pre-warm the required amount of Complete ExCellerate Human T Cell Media to room temperature.
2. Isolate T cells using desired protocol.
3. Add 10 mL of pre-warmed complete media to wells of G-Rex®6M Well Plate. Add activating reagent per manufacturer's recommendations.
4. Plate 5 x 10^6 cells/well of $CD3^+$ T cells.

Day 2 or 3 (depending on visual cell confluency)

5. Add 90 mL of fresh pre-warmed complete media to each well of G-Rex®6M Well Plate.

Day 8 or 9

6. Harvest the cells on Day 8 or 9 or when cell confluency exceeds 30 x $10^6/cm^2$ for desired downstream applications.

RECOMMENDED PROTOCOL (T75 Flasks)

Day 0

1. Pre-warm the required amount of Complete ExCellerate Human T Cell Media to room temperature.
2. Isolate peripheral blood mononuclear cells (PBMCs) using desired protocol.
3. Dilute antibodies into 1X PBS for a final concentration of 1 µg/mL Anti-CD3, and 3 µg/mL Anti-CD28. Add 1 mL of antibody solution/well of a 24-well plate.
4. Incubate plate at 37 °C and 5% CO_2 in a humidified incubator for at least 60 minutes. After incubation, rinse 24-well plate 2X with 1 mL fresh 1X PBS.
5. Dilute cell suspension to 0.5 x 10^6 cells/well in pre-warmed Completed ExCellerate Human T Cell Media containing desired cytokines. Add 2 mL/well of a 24-well plate (final seeding density of 1 x 10^6 cells/well).
6. Incubate the cells 37 °C and 5% CO_2 in a humidified incubator.

Day 3

7. Transfer the cell suspension to a T75 flask and add 10 mL of pre-warmed Completed ExCellerate Human T Cell Media.
   Note: *Cells may stick to bottom and sides of the well. Agitate cells somewhat vigorously to detach from well.*

Day 8

9. Harvest the cells for desired downstream applications.