

# CelThera™ GMP T Cell Expansion Medium

## Product Overview

CelThera™ GMP T Cell Expansion Medium is a serum-free, animal origin-free medium produced under GMP conditions to support *in vitro* culture of human T cells. Animal origin-free media avoids the risk of introducing pathogenic microorganisms and can improve batch-to-batch consistency over conventional and Xeno-free media. CelThera™ GMP T Cell Expansion Medium is designed for large scale expansion of T cells, which eliminates the need for serum or serum replacements. If users decide to add serum or serum replacements into the medium, the amount required needs to be determined by the specific T cell applications. CelThera™ GMP T Cell Expansion Medium contains only recombinant proteins as components, and no antibiotics included in formulation.

## Product Specifications

Cat. No.	Contents	Amount	Storage	Shelf life
GMP-CM3101	CelThera™ GMP T Cell Expansion Medium	1000ml	2°C-8°C. Protect from light	18 months
GMP-CM3101-1	CelThera™ GMP T Cell Expansion Supplement	7.25ml	-20°C or below. Protect from light	12 months

## Handling / Directions For Use

### 1. Preparation of Complete Medium

Thaw CelThera™ GMP T Cell Expansion Supplement (Cat. No. GMP-CM3101-1) to room temperature. In a biosafety cabinet, remove the lid on both CelThera™ GMP T Cell Expansion Supplement and CelThera™ GMP T Cell Expansion Medium (Cat. No. GMP-CM3101). Add 7mL of supplement into 1L of expansion medium and close the lid. Mix upside down 3 to 5 times and store the mixed complete culture medium at 2°C to 8°C. It is recommended to use the mixed complete culture medium within 4 weeks.

*\*Please note: this complete medium does NOT contain cytokines. For T cell expansion, additional cytokines such as IL-2, IL-7, or IL-15, are needed. The amount of cytokines or other growth factors needed will vary depending on your specific T cell application.*

### 2. Cell Culturing

- Prepare fresh PBMCs or rapidly thaw frozen PBMCs in a 37°C water bath according to a standard PBMCs thawing protocol.

- Wash cells with PBS or DPBS as needed. Centrifuge PBMCs at 200-300 x g for 5 to 10 minutes and remove wash buffer.
- Aliquot an appropriate volume of complete medium and add IL-2 into the medium.
- Resuspend PBMCs in appropriate amount of complete medium containing IL-2 and count cell number and viability.
- Activate T cells for subsequent expansion by using T cell activators, such as stimulatory antibodies (e.g., CD3/CD28 monoclonal antibodies), antibody-conjugated magnetic beads, or antibody-conjugated biodegradable nanobeads.
- Depending on the number of viable PBMCs, adjust the density to  $1 \times 10^6$  cells/mL using complete medium containing IL-2. Inoculate 3 to 4 mL per well in a 6 well-plate and place into an incubator with 5% CO<sub>2</sub> at 37°C.
- On Day 2, replenish each well with 3 to 4 mL of complete medium containing IL-2.
- On Day 4, passage cells from the 6-well plate to a T25 flask. First, adjust the density to  $3-4 \times 10^5$  cells/mL using complete medium containing IL-2. In each T25 flask, inoculate with 10 to 12 mL and passage cells every 2-3 days. Please note that T cell growth rates may vary from different donors.

### Special Notes

1. T cells grow logarithmically between Day 4 to 10, so it is recommended to passage T cells at a low density every 2 days in this period for the optimal expansion rate. After Day 10, increase the cell inoculation density (e.g.  $6-8 \times 10^5$  cells/mL) and passage cells every 2-3 days to obtain a higher expansion rate.
2. For high-density culture in bioreactors, such as the WAVE Cell Bag™ Bioreactor, the optimal procedure needs to be determined through prior experience or experimental results.

### Related Products

- GMP Human IL-2 Protein (Cat. No. GMP-L02H14)
- GMP Human IL-7 Protein (Cat. No. GMP-L07H24)
- GMP Human IL-15 Protein (Cat. No. GMP-L15H13)
- GMP Human IL-21 Protein (Cat. No. GMP-L21H25)
- GMP Monoclonal Anti-Human CD3 Antibody (OKT3) (Cat. No. GMP-MC0323)
- GMP Monoclonal Anti-Human CD28 Antibody (Cat. No. GMP-MC2824)
- GMP Human 4-1BB Ligand Protein, Fc Tag (Cat. No. GMP-41LH26)
- GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads (Cat. No. GMP-MBS001)