

# Manual Instructions

## Product Overview

$\gamma\delta$ T Cell Expansion Serum free Medium is a serum free, animal component free, and antibiotic free medium used for culturing, maintenance, and expansion of  $\gamma\delta$ T cells. Compared to the medium containing serum or component derived from serum, the serum free medium significantly reduces the risk of introducing heterologous substances to the  $\gamma\delta$ T cell culture process. In addition, the batch to batch consistency of the medium is more stable than that of the serum containing medium, improving the batch to batch uniformity of the medium. When the Cell Expansion Serum free Medium is used in combination with heat inactivated autologous plasma or human AB serum, the expansion effect of the medium can be greatly enhanced.

## 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
CM31S3	CelThera™ Immune Cell Supplement D	1.5ml	20°C or below. Protect from light	12 months

## Handling / Directions For Use

Preparation of  $\gamma\delta$ T Complete Medium:

Thaw the CelThera™ GMP T Cell Expansion Medium, CelThera™ GMP T Cell Expansion Supplement and CelThera™ Immune Cell Supplement D at room temperature, open the lid of the basal medium and the lid of the supplement in a biosafety cabinet. Add 7 ml of CelThera™ GMP T Cell Expansion Supplement and 1.5 ml of CelThera™ Immune Cell Supplement D to 1000 mL of CelThera™ GMP T Cell Expansion Medium. mix upside down for 3~5 times. The Complete Medium can be stored at 2 - 8 °C for 3 - 4 weeks.

Activation and Expansion of  $\gamma\delta$ T Cells:

Take PBMC and AB serum as starting material as an example

1. On day 0, resuspend PBMC with  $\gamma\delta$ T complete medium (containing 10% AB serum) supplemented with zoledronic acid at a final concentration of 6 - 7  $\mu$ M and IL - 2 at a final concentration of 100 - 1000 IU/ml. Seed PBMC into a culture well plate or culture flask (PBMC seeding density: 3 - 5 $\times$ 10<sup>6</sup> cells/mL is recommended). Then place it in an incubator at 37 °C with 5% CO<sub>2</sub>.
2. On day 2, add  $\gamma\delta$ T complete medium (containing 10% AB serum) supplemented with IL - 2 at a final concentration of 100 - 1000 IU/ml equivalent to twice the amount on day 0 into the culture well plate or culture flask. For example, if 1 ml of medium was used when inoculating PBMC on day 0, add 2 ml of medium on day 2.
3. On day 5, count cell number and viability, add  $\gamma\delta$ T complete medium (containing 10%

AB serum) supplemented with IL - 2 at a final concentration of 100 - 1000 IU/ml, adjust the cell density to  $2 - 3 \times 10^5$  cells/mL, and passage into wells or flasks according to the volume of the cell suspension.

4. On day 7, count cell number and viability, add  $\gamma\delta$  T complete medium (containing 5% AB serum) supplemented with IL - 2 at a final concentration of 100 - 1000 IU/ml, adjust the cell density to  $2 - 3 \times 10^5$  cells/mL, and passage into wells or flasks according to the volume of the cell suspension.
5. From day 9, passage cells each 3 days, count cell number and viability, add  $\gamma\delta$  T complete medium (containing 1 - 2% AB serum) supplemented with IL - 2 at a final concentration of 100 - 1000 IU/ml, adjust the cell density to  $2 - 3 \times 10^5$  cells/mL, and passage into wells or flasks according to the volume of the cell suspension.
6. Harvest the cells on days 12 - 14.

**Special Notes:**

The medium should be equilibrated to room temperature before use.