

# **ExCell Bio**

## OptiVitro® T Cell Serum-free Medium

For Research and Manufacturing Use Not Intended for Diagnostic and Therapeutic Use

## **User Manual**

Catalog Number TE000-N022
TE000-N021
TE000-N021S





### | Product description

OptiVitro® T Cell Serum-free Medium is a serum-free, xeno-free medium that has been specifically designed to expand human T lymphocytes *in vitro*. The medium consists of two components: OptiVitro® T Cell Basal SF Medium and OptiVitro® T Cell SF Medium Supplement, both of which are sterile and manufactured in compliance with GMP regulations. The product is free of cytokines and antibiotics.

## Contents and storage

Catalog No.	Product name	Amount	Storage	Shelf life <sup>[1]</sup>
TE000-N022	OptiVitro® T Cell Serum-free Medium	1 kit	-	-
BA0032	OptiVitro® T Cell Basal SF Medium	1000 mL	2-8 °C Protect	12 months
			from light	
BA0052	OptiVitro® T Cell SF Medium Supplement	8 mL	2-8 °C Protect	18 months
			from light	
TE000-N021	OptiVitro® T Cell Serum-free Medium	1 kit	-	-
BA0031	OptiVitro® T Cell Basal SF Medium	500 mL	2-8 °C Protect	12 months
			from light	
BA0051	OptiVitro® T Cell SF Medium Supplement	4 mL	2-8 °C Protect	18 months
			from light	
TE000-N021S	OptiVitro® T Cell Serum-free Medium	1 kit		
	(Sample)		-	-
BA0031S	OptiVitro® T Cell Basal SF Medium (Sample)	100 mL	2-8 °C Protect	12 months
			from light	
BA0051S	OptiVitro® T Cell SF Medium Supplement	0.8 mL	2-8 °C Protect	18 months
	(Sample)		from light	

<sup>[1]</sup> The Shelf-Life may be extended after strict validation by QC.

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#### Instructions for use

#### Prepare media

- 1. Place OptiVitro® T Cell Basal SF Medium and OptiVitro® T Cell SF Medium Supplement in a sterile laminar flow hood.
- 2. Add 4 mL of OptiVitro® T Cell SF Medium Supplement to 500 mL of OptiVitro® T Cell Basal SF Medium, or 8 mL of OptiVitro® T Cell SF Medium Supplement to 1000 mL of OptiVitro® T Cell Basal SF Medium.
- 3. Tighten the cap and mix the OptiVitro® T Cell Basal SF Medium thoroughly.
- 4. The complete OptiVitro® T Cell Basal SF Medium can be supplemented with cytokines like IL-2, IL-7, or IL-15 to support T-cell expansion.

Note: It is recommended to use complete OptiVitro® T Cell Serum-free Medium within four weeks after mixed.

#### **Culture T-cells from PBMCs**

- 1. Prepare fresh peripheral blood mononuclear cells (PBMCs) according to standard separation protocols, or rapidly thaw (<1 minute) frozen vials of PBMC cells in a 37°C water bath.
- 2. If using freshly prepared PBMCs, they can be directly used after washing with sterile DPBS. For frozen cells, it is recommended to thaw them one day prior to T-cell activation, and incubate them in complete OptiVitro® T Cell Serum-free Medium without extra cytokines at a concentration of approximately 2×10<sup>6</sup> cells/mL, in a humidified 37°C incubator with an atmosphere of 5% CO<sub>2</sub> for 16-24 hours.
- 3. Centrifuge the cells at 400×g for 10 minutes and remove the supernatant.
- 4. Before use, equilibrate complete OptiVitro® T Cell Serum-free Medium to room temperature. Resuspend PBMCs at a concentration of 0.5-1×10<sup>6</sup> cells/mL in complete OptiVitro® T Cell Serum-free Medium supplemented with cytokines such as IL-2, IL-7, or IL-15.
- Transfer the cells to culture plates that are pre-coated with anti-human CD3/CD28 antibodies for activating
   T-cells to initiate the culture, or use commercially available beads according to the manufacturer's protocol.
- 6. Incubate the cells in a humidified 37°C incubator with an atmosphere of 5% CO<sub>2</sub>.
- 7. Feed and adjust the cell concentration to 0.5-1×10<sup>6</sup> cells/mL with complete OptiVitro® T Cell Serum-free Medium supplemented with cytokines every 2-3 days. The cells can be transferred to bioreactors for further expansion at around Day 7 after T-cell activation.

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