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ExCell

ExCell Bio

OptiVitro[®] T Cell Serum-free Medium (phenol red-free)

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

User Manual

Catalog Number TE000-N032 TE000-N031 TE000-N031S

> TE000-N071 TE000-N072 TE000-N073



Product description

OptiVitro[®] T Cell Serum-free Medium (phenol red-free) 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 (phenol red-free) 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 ^[1]
TE000-N032	OptiVitro [®] T Cell Serum-free Medium (phenol red-free)	1 kit	-	-
BA0042	OptiVitro [®] T Cell Basal SF Medium (phenol red-free)	1000 mL (bottle)	2-8 °C Protect from light	12 months
BA0052	OptiVitro [®] T Cell SF Medium Supplement	8 mL 2-8 °C Protect from light		18 months
TE000-N031	OptiVitro [®] T Cell Serum-free Medium (phenol red-free)	1 kit	-	-
BA0041	OptiVitro [®] T Cell Basal SF Medium (phenol red-free)	500 mL (bottle)	2-8 °C Protect from light	12 months
BA0051	OptiVitro [®] T Cell SF Medium Supplement	4 mL	2-8 °C Protect from light	18 months
TE000-N031S	OptiVitro® T Cell Serum-free Medium (phenol red-free) (Sample)	1 kit	-	-
BA0041S	OptiVitro [®] T Cell Basal SF Medium (phenol red-free) (Sample)	100 mL (bottle)	2-8 °C Protect from light	12 months
BA0051S	OptiVitro [®] T Cell SF Medium Supplement (Sample)	0.8 mL	2-8 °C Protect from light	18 months
TE000-N071	OptiVitro [®] T Cell Serum-free Medium (phenol red-free)	1 L (bag)	-	-
BA0161	OptiVitro [®] T Cell Basal SF Medium (phenol red-free)	1 L (bag)	2-8 °C Protect from light	12 months
BA0171	OptiVitro [®] T Cell SF Medium Supplement	8 mL 2-8 °C Protect from light		18 months

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Catalog No.	Product name	Amount	Storage	Shelf life ^[1]
TE000-N072	OptiVitro® T Cell Serum-free Medium (phenol red-free)	2 L (bag)	-	-
BA0162	OptiVitro [®] T Cell Basal SF Medium (phenol red-free)	2 L (bag)	2-8 °C Protect from light	12 months
BA0172	OptiVitro [®] T Cell SF Medium Supplement	16 mL	2-8 °C Protect from light	18 months
TE000-N073	OptiVitro® T Cell Serum-free Medium (phenol red-free)	5 L (bag)	-	-
BA0163	OptiVitro [®] T Cell Basal SF Medium (phenol red-free)	5 L (bag)	2-8 °C Protect from light	12 months
BA0173	OptiVitro [®] T Cell SF Medium Supplement	40 mL	2-8 °C Protect from light	18 months

^[1] The Shelf-Life may be extended after strict validation by QC.

Instructions for use

Prepare media

• Bottle format:

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

• Bag format:

- Place OptiVitro[®] T Cell Basal SF Medium (phenol red-free) and OptiVitro[®] T Cell SF Medium Supplement in a sterile laminar flow hood.
- 2. Open the cap of OptiVitro® T Cell SF Medium Supplement and draw the liquid using a sterile syringe.
- 3. Inject the liquid into the bag of OptiVitro[®] T Cell Basal SF Medium (phenol red-free) through the needle sampling port of Tube 2 (refer to the diagram below), or through the Male Luer taper of Tube 3.
- 4. Mix the contents thoroughly by shaking the bag.
- 5. The complete OptiVitro[®] T Cell Serum-free Medium (phenol red-free) can be supplemented with cytokines like IL-2, IL-7, or IL-15 to support T-cell expansion.

∩ Tube 2	Tube	Description	Remark	
Tube 3	Tube 1	C-flex tubing, sealed end	/	
Tube 14.	Tube 2	Silicone tubing with needle sampling port	For sterile sampling	
	Tube 3	0.122"ID x 0.161"OD PVC tubing with Male Luer taper and Female cap	For connecting culture bags	
	Tube 4	0.122"ID x 0.161"OD PVC tubing with Female Luer taper and Male cap	through Luer taper or PVC welding	

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• Note: It is recommended to use complete OptiVitro[®] T Cell Serum-free Medium (phenol red-free) within four weeks after mixed.

Culture T-cells from PBMCs

- 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 (phenol red-free) without extra cytokines at a concentration of approximately 2×10⁶ cells/mL, in a humidified 37°C incubator with an atmosphere of 5% CO₂ for 16-24 hours.
- 3. Centrifuge the cells at $400 \times g$ for 10 minutes and remove the supernatant.
- 4. Before use, equilibrate complete OptiVitro[®] T Cell Serum-free Medium (phenol red-free) to room temperature. Resuspend PBMCs at a concentration of 0.5-1×10⁶ cells/mL in complete OptiVitro[®] T Cell Serum-free Medium (phenol red-free) 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% CO₂.
- 7. Feed and adjust the cell concentration to 0.5-1×10⁶ cells/mL with complete OptiVitro[®] T Cell Serum-free Medium (phenol red-free) 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.