BI
Biological Industries
Culture of Excellence

BIOTARGET™

Serum-free, xeno-free medium, optimized specifically for activation and expansion of T cells

Cat. No.: 05-080-1A 500ml

Instructions for Use

Product Description

BIOTARGET™ is a chemically defined, xeno-free, serum-free medium for in-vitro human T cell activation and expansion for human in vitro cell culture applications.

Features

- Defined, serum-free (SF), xeno-free (XF) medium.
- The proteins that are used are human albumin, human transferrin and recombinant human insulin.
- Does not contain glutamine and antibiotics.
- Manufactured under cGMP conditions.
- For the activation and expansion of various T cell populations such as Peripheral Blood Mononuclear Cells (PBMC), gene-modified T cells, Tumor Infiltrating Lymphocytes (TIL) and T cells encoding recombinant receptors (CAR-T).
- Recombinant cytokines, required for the optimal growth and expansion of T cells, have not been added to BIOTARGET™. This allows users the flexibility to prepare final medium that meets their requirements, such as addition of IL-2.
- Sterile by aseptic processing and filtration.

Precaution and Disclaimer

1. Do not use if a visible precipitate is observed in the medium.
2. Do not use BIOTARGET™ medium beyond the expiration date indicated on the product label.
3. To maintain sterility use aseptic techniques.

Storage and Stability

BIOTARGET™ should be stored at 2-8˚C.
Protect the medium from light.
Shelf Life: Refer to product label for expiration date.

Instructions for Use

Medium preparation

BIOTARGET™ medium requires supplementation with 4mM L-glutamine, heat inactivated human serum (HS) type AB (5-10%) and cytokines such as IL-2 to support T-cell expansion. It is recommended to use 30–300 IU/ml of human recombinant IL-2 for standard T cell expansion. The amount of IL-2 may vary depending on experimental conditions. Medium can be further supplemented with antibiotics if desired.

T Cell Culture

This protocol is a general guideline applicable of Peripheral Blood Mononuclear Cells (PBMC) and isolated T cells.

1. Prepare PBMC according to standard protocols (e.g. by Ficoll density gradient centrifugation) or rapidly thaw frozen cells at 37°C.
2. Wash cells with Dulbecco's Phosphate Buffered Saline (DPBS) without calcium and magnesium. DPBS may be supplemented with 2–5% heat-inactivated human type AB serum.
3. Determine viable cell concentration.
4. Centrifuge cells at 200 × g for 5–10 minutes and aspirate the supernatant.
5. Resuspend PBMC pellet at approximately 0.5–1 × 10^6 cells/ml in medium supplemented with cytokines (e.g., IL-2).
6. Transfer the desired number of cells to the desired tissue culture vessel.
7. Add stimulants (inactivated antigen presenting cells (APC) or stimulatory antibodies (such as CD3/CD28) as coated magnetic beads or soluble molecules): See below.
8. Incubate the culture vessel at 37°C in a humidified atmosphere with 5% CO_2.
9. Count the cells at least twice a week a d adjust cell density to 0.5-1x10^6 cells/ml with the addition of fresh medium supplemented with cytokines to maintain log phase growth.

Activation of PBMC or isolated T cells

The following is a general guideline for T cell activation and expansion. Cells can be activated and expanded using mitogens, irradiated allogenic feeder cells, or other T cell receptor antibodies. In each case, use according to the manufacturer instructions. Optimization of the expansion procedures may be needed depending on culture system and aplications (e.g. activation method and reagents, cell seeding density and cytokine concentration).

Coating Procedure for T cell Activation

1. Coat culture vessel with anti-human CD3 and anti-human CD28 antibodies at 1µg/ml in DPBS for 2 hours at 37°C or overnight at 2-8°C. Cover with Parafilm® to prevent evaporation.
2. Aspirate coating solution and discard.
3. Wash culture vessel twice with DPBS before the addition of cells.

T cell Activation using CD3/CD28 beads or soluble antibodies

Start with purified human T cells at 0.5-1 x 10^6 cells/ml in BIOTARGET™ Medium supplemented with 5-10% heat inactivated human serum and cytokines e.g. 30-300 IU/ml Human Recombinant IL-2.

1. Activate human T cells with human CD3/CD28 beads (e.g. Dynabeads™ Human T-Activator CD3/CD28) or with soluble antibodies (e.g ImmunoCult™ T Cell Activator). Incubate cells at 37°C and 5% CO_2.
2. Count the cells at least twice a week and adjust the cell density to 0.5-1 x 10^6 cells/ml with the addition of fresh medium supplemented with cytokines.
3. For long-term expansion of human T cells, harvest and resuspend the expanded T cells at 0.5-1x10^6 cells/ml in fresh culture medium and re-stimulate every 7-10 days.

Quality Control

BIOTARGET™ performance is tested for optimal maintenance and expansion of cells. Additional standard tests are pH, osmolality, appearance and sterility tests.

Auxiliary Products:

Product	Cat. No.
Dulbecco's Phosphate Buffered Saline w/o Calcium and Magnesium (DPBS)	02-023-1
L-glutamin solution, 200mM	03-020-1
NutriFreez™ D10 Cryopreservation Medium	05-713-1

Quality Assurance

- For in vitro diagnostic use, research use or for use as ancillary material in manufacturing Cell-Gene or Tissue-based products.
- Notified under US FDA IVD part 864.9225 Cell-freezing apparatus and reagents for in vitro diagnostic use.
- Listed in Europe under CE IVD class I, thus comply with European In-Vitro Diagnostic Devices Directive (98/79/EC) requirements.
- Manufactured under ISO 13485 QMS and in compliance with applicable cGMP guidelines.
- Manufactured under controlled environments and processes in accordance with:
  - ISO 13408 – Aseptic Processing of Health Care Products;
  - ISO 14644 – Airborne Particulate Cleanliness Classes in Clean Rooms and Clean Zones;

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Product Label Symbols

	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.
	The product meets the requirements of the applicable EC directives
	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	Indicates a medical device that has been manufactured using accepted aseptic techniques.