Instructions for Use

4Cell® Nutri-T Medium

A xeno-free medium to support the expansion of human PBMC's, CAR-T and TILs, without the addition of serum

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1. Introduction

Adoptive immunotherapy of malignant diseases using patient-derived T cells have attracted significant interest due to the positive results from clinical trials. This includes the activation and expansion of various T cell populations, e.g. Tumor Infiltrating Lymphocytes (TILs) and Chimeric Antigen Receptor T cell (CAR-Ts).

CAR-T cell therapy is based on autologous or allogeneic lymphocytes, engineered to express a chimeric antigen receptor (CAR). The engineered lymphocytes are expanded ex-vivo followed by injection to the patient(s).

TIL therapy consists of isolating a patient's own naturally occurring TILs from a tumor biopsy, extraction of TILs from the tumor, activation by antigen presenting cells or CD3/CD28 antibodies, ex-vivo expansion followed by injection back to the patient(s).

4Cell® Nutri-T Medium is a xeno-free medium, optimized for these aforementioned applications involving human T cell activation and expansion in ex-vivo cell culture conditions.

Features

- Complete medium
- xeno-free (XF) medium
- Does not require the addition of serum
- Contains Human Serum Albumin (HSA)
- Contains Stable L-Alanyl-L-Glutamine
- Does not contain antibiotics
- The medium may require the addition of cytokines according to the specific applications

Precaution and Disclaimer

- For research use only. Not for therapeutic use
- Do not use the medium if a visible precipitate is observed

Storage and Stability

- Store at 2-8°C.
- Protect medium from direct light.
- Refer to product label for date of manufacture

2. Instructions for Use

2.1 Medium Preparation

To support T cell expansion in 4Cell® Nutri-T medium, a supplementation with appropriate cytokines is required. For standard T cell expansion, it is recommended to use 300 – 3000 IU/ml of rhIL-2. The amount of Human Recombinant IL-2 may vary depending on experimental conditions.

2.2 General T Cell Culture

This protocol is a recommended general guideline applicable for Peripheral Blood Mononuclear Cells (PBMCs) and isolated T cell;

1. Isolate PBMCs according to standard protocols (e.g. by Ficoll density gradient centrifugation); Wash cells with Dulbecco's Phosphate Buffered Saline (DPBS) without calcium and magnesium. Alternatively, thraw frozen cells in 4Cell® Nutri-T medium at 37 °C.
2. Determine viable cell concentration.
3. Centrifuge cells at 200 × g for 5 – 10 minutes and aspirate the supernatant.
4. Resuspend PBMCs pellet at approximately at 0.1 – 1 × 10^6 cells/ml in medium supplemented with cytokines (e.g. IL-2).
5. Transfer the desired number of cells to the tissue culture vessel.
6. Add stimulants (e.g. 1:100 TransAct™ or preferred activation agent)

2.3 Activation of PBMCs 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, activate the cells according to the manufacturer's instructions.**
Optimization of the expansion procedures may be needed depending on culture system and applications (e.g. activation method and reagents, cell seeding density and cytokine concentration).

Case 1: T cell Activation using TransAct™
Start with purified human T cells at 0.1 – 1 × 10^6 cells/ml in 4Cell® Nutri-T with cytokines (e.g. 600 IU/ml Human Recombinant IL-2).
Add 1:100 of TransAct to the initial seeding.

Case 2: T cell Activation using CD3/CD28 beads or soluble antibodies
* Dynabeads™ Human T-Activator CD3/CD28 (1:1 beads/cells ratio is recommended)
* Soluble antibodies (e.g ImmunoCult™ T Cell Activator).

Start with purified human T cells at 0.1 – 1 × 10^6 cells/ml in 4Cell® Nutri-T and cytokines (e.g. 600 IU/ml Human Recombinant IL-2).

General Approach for Activation and Expansion

Day 0:

1. Start with purified human T cells at 0.1–1 × 10^6 cells/ml in 4Cell® Nutri-T and cytokines (e.g. 600 IU/ml Human Recombinant IL-2)
2. Activate human T cells with the activator of interest per the case samples above(this step is only needed at initial seeding)

Days 5–14 (or longer if desired):

3. Count the cells at least twice a week and adjust the cell density to 0.1–1 × 10^6 cells/ml with the addition of fresh medium supplemented with cytokines.
4. For long-term expansion of human T cells, harvest and resuspend the expanded T cells at 0.5–1 × 10^6 cells/ml in fresh culture medium and re-stimulate every 7–10 days.

⚠ The cell's seeding concentration may vary according to the desired application and | or manufacturer's instructions.

2.4 CAR-T Culture

Day 0:

1. Isolate PBMCs according to standard protocols (e.g. by Ficoll density gradient centrifugation) or rapidly thaw frozen cells into 4Cell® Nutri-T medium at 37°C.
2. Centrifuge cells at 200 × g for 5 – 10 minutes and aspirate the supernatant.
3. Prepare medium by supplementation with IL-2 (300 IU/ml) and OKT-3 (50 ng/ml).
4. Gently re-suspend PBMC pellet in pre-warmed medium supplemented with OKT3 and IL-2.
5. Transfer the desired number of cells (e.g. of 1×10^6/ml cells)) to the appropriate cultureware.
6. Incubate culture vessel at 37°C in a humidified atmosphere with 5% CO2.

Day 2:

7. Adjust cells to 0.5×10^6 cells/ml in medium containing 300 IU/ml IL-2 w/o OKT-3. Add fresh medium as needed to reach this concentration level
8. Perform transduction according to manufacturer's protocols.

Days 3 – 10:

9. Perform viable cell count and split as needed to maintain optimal growth. The split should be considered when the cell count reaches $>1 \times 10^6$ cells/ml. No need to add additional OKT-3
10. Perform 50% medium change and refresh IL-2 every 2-3 days. IL-2 should only be added to the fresh medium.

Day 6 or preferred:

11. FACS analysis may be performed according to preferable calibrated protocols for CAR-T expression and T cell markers (e.g. CD3, CD4, CD8).

Day 10:

11. Perform viable cell count. FACS analysis may again be performed according to preferable calibrated protocols (e.g., CAR-T, CD3, CD4, CD8).

2.5 Large Scale Rapid Expansion Protocol (REP) of TIL in G-REX® 100 (450 ml)

Day 0:

1. Supplement medium with IL-2 (e.g. 3000 IU/ml), OKT-3 (e.g. 30 ng/ml) and feeder cells (e.g. irradiated PBMCs) (1 : 100 TILs : feeders) or other activation methods.
2. Seed TILs (5×10^6/ml) and add 4Cell® Nutri-T medium (for G-REX® 100 final volume of 400 ml)

Day 5:

3. Perform medium change, aspirate 250 ml cell-free medium and replenish with 300 ml fresh medium with IL-2. No need to add additional OKT-3

Day 7 – 8:

4. Perform viable cell count. Splitting of the culture should be done when cells reach 1×10^6 cells/ml
5. When splitting, adjust cell concentration to $0.3 – 0.5 \times 10^6$ cells/ml in subsequent flasks.

Day 11:

6. Perform viable cell count, change medium (e.g. remove 300 ml of cell-free medium by aspiration and add 300 ml fresh media with IL-2, e.g. 3000 IU/ml).

Day 14:

7. Perform viable cell count. FACS analysis may again be performed according to the preferred protocol. Analyze and subsequently use cells for the intended application

3. Quality and Manufacturing

3.1 Quality Control

4Cell® Nutri-T is performance tested for optimal maintenance and expansion of cells. Additional standard tests are pH, Osmolality, Endotoxin, Mycoplasma, appearance, and sterility tests.

3.2 Quality Assurance

For research use in development of Cell, Gene, or Tissue-based products.
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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3.3 Product Label Symbols

Indicates a product that has been manufactured using accepted aseptic techniques


Consult the IFU (instructions for use) for further product information and detail

The information and figures contained in these instructions correspond to the version date specified below.
Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice. Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote the other gender as well.

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