Instructions for Use

4Cell® Nutri-T GMP Medium

Xeno-free medium for the cultivation of lymphocytes,
for ex-vivo use

2979876-000

SARTORIUS

Product	Cat. No
4Cell® Nutri-T GMP Medium with Phenol Red	05-F3F2111-1K
4Cell® Nutri-T GMP Medium without Phenol Red	05-F3F2101-1K

Contents

1 Introduction

Adoptive immunotherapies 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 cells (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 GMP Medium medium is a defined xeno-free medium, optimized for ex-vivo expansion of Human PBMCs and T cells that may be used in the aforementioned applications. To address the specific needs of your process, 4Cell® Nutri-T GMP Medium is available in both phenol red-containing and phenol red-free standard versions.

1.1 Features

- Complete medium
- Xeno-free (XF) medium
- Serum-free (SF) 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.

1.2 Intended Use and Safety

- GMP version is intended for research use and use as ancillary material in ex vivo cell processing only.
- **Not** intended for human in vivo applications.
- Do **not** use the medium if a visible precipitate is observed.
- Do **not** use in case of change of color.
- Do **not** use beyond the expiration date indicated on the product label.
- Protect the medium from direct light.

1.3 Storage and Stability

- Store at 2 – 8 °C.
- **Must** be warmed to room temperature (15 °C – 30 °C) before use.
- To ensure stability of the medium, warm only the amount needed.
- Protect medium from direct light.
- Shelf life: Refer to product label for expiration date.

2 Instructions for Use

2.1 Medium Preparation

To support T cell expansion in **4Cell® Nutri-T** medium, a supplementation with appropriate cytokines may be required. For standard T cell expansion, it is recommended to use 100 – 3000 IU/mL of recombinant human (rh) IL-2. The amount of rhIL-2 may vary depending on experimental conditions. Alternatively, rhIL-2 may be replaced with rhIL-7 and rhIL-15 at concentration of 10 ng/L each.

When using the same medium bottle for longer than 2 weeks, or if less than 400 mL of liquid medium remaining in the provided 1 L bottle, transfer the remaining medium to a smaller bottle or bottles (aliquoting), so the amount of headspace relative to the stored medium volume is minimized, thus avoiding pH elevation.

If required, antibiotics may be added at 50 % lower concentration than the common concentration used (e.g. 0.5 % rather than 1 % for final concentration of: Penicillin 50 units/mL, Streptomycin 50 ug/mL, Amphotericin 0.125 ug/mL). Higher conentrations are **not** recommended and may reduce cell proliferation in SF culture condition.

2.2 General T Cell Culture – Initial Cells Seeding

This protocol is a recommended general guideline applicable for Peripheral Blood Mononuclear Cells (PBMCs) and isolated T cells (e.g. by Ficoll density gradient centrifugation).

Day 0 (for frozen cells):

1. Thaw cells quickly in a 37 °C bath.
2. Thawed cells should be centrifuged prior to cells seeding to eliminate DMSO residues (150 – 200 g, for 5 – 10 minutes) followed by resuspension in warm (37 °C) Nutri-T.
3. Perform viable cells count.
4. Transfer the desired number of cells to a tissue culture vessel containing Nutri-T medium, for 24 hrs (for cells recovery post thawing or isolation). Recommended cells seeding concentration 0.1 – 1 x 10^6 cells/mL.

   NOTE Neither activators nor cytokines are required for the 24 hrs recovery step.

Day 1 (for isolated PBMCs, purified T cells after apheresis, and thawed cells):

1. Perform viable cells count and seed the desired number of cells to a tissue culture vessel. Recommended cells seeding concentration 0.1 – 1 x 10^6 cells/mL. If required, centrifugate the cells (150 – 200 g, for 5 – 10 minutes) followed by resuspension in Nutri-T.

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

2. Add cytokines (e.g. 300 IU/mL rhIL-2).
3. Add Activator (refer to chapter 2.2.1, page 7).

2.2.1 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 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™

- Add 1:100 of TransAct™ at 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)

  NOTE Activator should be added only once, at the initial seeding.

Days 3 – 14 (or longer if desired):

From day 3 of culture, every 2 – 3 days perform viable cells count and split as required, (0.1 – 1 x 10^6 cells/mL is recommended to maintain the cells at logarithmic growth). When no split is required, perform 50 % medium change (aspirate 50 % cell-free medium and replenish with 50 % fresh medium supplemented with fresh cytokines (e.g. 300 IU/mL rhIL-2).

NOTE

- Split should be considered when the cell density reaches >1 x 10^6 cells/mL.
- At each treatment add fresh cytokines (recommended every 2 – 3 days).
- Using SF culture medium, 50 % medium changes are more recommended than on-top medium addition.
- No need to add additional activator.

2.3 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 x g for 5 – 10 minutes and aspirate the supernatant.
3. Prepare medium by supplementation with rhIL-2 (300 IU/mL) and OKT-3 (50 ng/mL).
4. Gently re-suspend PBMC pellet in pre-warmed medium supplemented with OKT-3 and IL-2.
5. Transfer the desired number of cells (e.g. 1 x 10^6/mL cells) to the appropriate cultureware .
6. Incubate culture vessel at 37 °C in a humidified atmosphere with 5 % CO_2.

Day 2:

7. Adjust cells to 0.5 x 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 cells count and split as needed to maintain optimal growth. Split should be considered when the cell count reaches >1 x 10^6 cells/mL. No need to add additional OKT-3.
10. Perform 50 % medium change and refresh IL-2 every 2 – 3 days. OKT-3 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:

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

2.4 Large Scale Rapid Expansion Protocol (REP) of TILs in G-REX® 100 (450 mL)

Day 0:

1. Supplement medium with rhIL-2 (e.g. 3000 IU/mL), OKT-3 (e.g. 30 ng/mL) and activators (e.g. irradiated PBMCs as feeder cells at 1:100 TILs:feeders, e.g. use 5 x 10^6/mL TILs and 500 x 10^6 feeder cells).
2. Seed TILs and add **4Cell® Nutri-T** medium (for G-REX® 100 final volume of 400 mL).

   NOTE TILs continue proliferating while the adherent irradiated feeder cells disappear as they die-off or get killed by the lymphocytes.

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 – 11:

4. Every 2 – 3 days perform viable cell count.
   - 4.1 Split the culture when cells reach 1 x 10^6 cells/mL. When splitting, adjust cell concentration to 0.3 – 0.5 x 10^6 cells/mL in subsequent flasks.
   - 4.2 If splitting is **not** required, change medium (e.g. remove 300 mL of cell-free medium by aspiration and add 300 mL fresh media with rhIL-2, e.g. 3000 IU/mL).

Day 14:

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

3 Quality

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.

For full specifications please check the lot specific Certificate of Analysis (CoA).

3.2 Quality Assurance

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.

Manufacturer
Biological Industries Israel Beit Haemek Ltd.
Kibbutz Beit Haemek 2511500, Israel

3.3 Product Label Symbols

REF	Indicates the manufacturer's catalogue number so that the product can be identified.
LOT	Indicates the manufacturer's batch code so that the batch or lot can be identified. **NOTE** Synonyms for batch code are lot number and batch number.

	Indicates the date after which the product is not to be used.
	Indicates the temperature limits to which the product can be safely exposed.
	Indicates a product that has been manufactured using accepted aseptic techniques.
	Consult the IFU (instructions for use) for further product information and detail.

4 Auxiliary Products

Product	Cat. No
4Cell® Nutri-T Medium (Research grade)	05-F3F211-1K
CellGenix® GMP rh IL-2	1020
CellGenix® Preclinical rh IL-2	1420
CellGenix® GMP rh IL-7	1010
CellGenix® Preclinical rh IL-7	1410
CellGenix® GMP rh IL-15	1013
CellGenix® Preclinical rh IL-15	1413
NutriFreez® D10 Cryopreservation Medium	05-713-01
NutriFreez® D5 Salt-Based Cryopreservation Solution	05-715-01

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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 all genders.

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