Instructions for Use

CellGenix® GMP T Cell Medium (TCM)

Protocol for T Cell Expansion in CellGenix® GMP TCM Using G-Rex® 10M or G-Rex® 6M

SARTORIUS

Contents

1 Safety

1.1 Intended Use

For research and further manufacturing use only.

2 Media Preparation

1. For one G-Rex®10M (or one G-Rex® 6M well plate), pre-warm 100 – 125 mL of CellGenix® GMP TCM to 37°C.
2. Supplement CellGenix® GMP TCM with CellGenix® IL-7 (10 ng/mL) and CellGenix® IL-15 (10 ng/mL). Antibiotics may be added as necessary e.g. Penicillin | Streptomycin 100 U/mL.
3. Transfer 95 mL of supplemented CellGenix® GMP TCM to the G-Rex® 10M.

3 Cell Preparation

3.1 Fresh Cells

1. Prepare fresh $CD3^+$ T cells using negative isolation, e.g. by EasySep™ Human T Cell Isolation Kit, Stemcell Technologies.
2. Wash cells in PBS to remove remaining EDTA from purification buffer.
3. Spin cells down at 300 x g, 21°C for 10 minutes. Carefully aspirate supernatant.
4. Resuspend cells in an appropriate volume of supplemented CellGenix® GMP TCM to a target density of 1×10^6 cells/mL. 5 mL of this single cell solution will be used for cell activation.

Optional: A 45 µm cell strainer may be applied to obtain a highly pure single cell solution.

3.2 Frozen Cells

1. Prepare 10 mL pre-warmed CellGenix® GMP TCM in a 15 mL conical tube.
2. Thaw one vial of purified $CD3^+$ T cells by stirring at 37°C for 1 minute in a water bath.
3. As soon as cells are thawed, quickly transfer cells to the prewarmed CellGenix® GMP TCM and invert the tube 3 times.
4. Spin cells down at 300 x g, 21°C for 10 minutes. Carefully aspirate supernatant.
5. Resuspend cells in an appropriate volume of supplemented CellGenix® GMP TCM to a target density of 1×10^6 cells/mL. 5 mL of this single cell solution will be used for cell activation.

Optional: A 45 µm cell strainer may be applied to obtain a highly pure single cell solution.

4 Cell Activation

1. Prepare cell activation beads with coupled anti- $CD3^+$ and anti-CD28 for 5×10^6 cells, e.g. Dynabeads Human T-Activator CD3/CD28 (Thermo Fisher) for a target ratio of 1:1 bead:cells.
2. Mix activation beads with 5 mL of $CD3^+$ T cells in supplemented CellGenix® GMP TCM (1×10^6 cells/mL) by pipetting up and down 3 times.
3. Add the whole volume of $CD3^+$ T cells with activation beads to the bottom of the prepared G-Rex® 10M containing 95 mL of supplemented CellGenix® GMP TCM. This results in a seeding density of 5×10^5 $CD3^+$ T cells/cm^2.
4. Incubate cells for 10 days* in a humidified incubator at 5% CO_2 at 37°C.

Optional: CellGenix® IL-7 (10 ng/mL) and CellGenix® IL-15 (10 ng/mL) may be supplemented every 2 – 4 days.

* A longer incubation may result in a higher cell yield while viability slowly decreases after 10 days in culture.

5 Cell Harvest

1. Carefully remove the G-Rex® 10M from the incubator (ensure that cells remain near the bottom membrane).
2. Aspirate 80 – 90 mL of the medium near the top of the liquid column.
3. Swirl the remaining medium to resuspend cells.
4. Transfer cells to your harvest container and determine cell number and viability.

Optional: Add 10 mL of fresh pre-warmed medium to the G-Rex® 10M to rinse and collect any residual cells.

Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen, Germany

Phone: +49 551 308 0
www.sartorius.com

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.

Last updated:
05 | 2023

LM | Publication No.: SCM6022-e230501