Instructions for Use

CellGenix® GMP TCM

Protocol for Lentiviral Transduction and Expansion of $CD3^+$ T Cells In CellGenix® GMP TCM

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1 Safety

1.1 Intended Use

For research and further manufacturing use only.

2 Media Preparation

1. For a 10-day cultivation in 96 well plate, 4 mL of CellGenix® GMP TCM is needed per donor.
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. Prepare fresh media with cytokines on each day of cultivation.

3 Cell Preparation

3.1 Fresh Cells

1. Prepare fresh $CD3^+$ T cells from apheresis or buffy coat 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 1×10^7 cells/mL. Cell suspension will be used for cell activation.

Optional: 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 by gently swirling the vial in the 37°C water bath until there is just a small clump of ice left in the vial.
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 1×10^7 cells/mL. Cell suspension will be used for cell activation.

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

4 Cell Activation and Seeding

NOTE
This protocol is designed to cultivate 3 wells for each medium and donor. If more replicates are required, the volumes have to be adjusted.

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1. Prepare anti- $CD3^+$ and anti-CD28 cell activation beads e.g. Dynabeads Human T-Activator CD3/CD28 (Thermo Fisher) for a target ratio of 1:1 bead:cells. Mix 9.75 µL beads with 350 µL supplemented CellGenix® GMP TCM to achieve a target ratio of beads:cells of 1:1 for 3.5×10^5 $CD3^+$ T cells.
2. Mix 315 µL of the bead | medium solution with 35 µL of the $CD3^+$ T cell suspension by pipetting up and down 3 times in a 1.5 mL reaction tube.
3. Transfer 100 µL of the $CD3^+$ T cells | activation bead suspension in 3 wells of a 96 well plate (flat bottom). This results in a seeding density of 1×10^6 $CD3^+$ T cells/mL.
4. Add 200 µL sterile PBS buffer in the surrounding wells to avoid media evaporation.
5. Incubate cells overnight in a humidified incubator at 5% CO_2 at 37°C.

5 Transduction: Day 1

NOTE
Viral safety precautions need to be followed.

1. Prepare pre-warmed CellGenix® GMP TCM supplemented with CellGenix® IL-7 (10 ng/mL) and CellGenix® IL-15 (10 ng/mL).
2. Add lentiviral particles to achieve the desired multiplicity of infection (MOI). For an MOI of 3 prepare 1.5×10^6 lentiviral particles in 0.5 mL medium. The added virus preparation should have a concentration of $> 6 \times 10^7$ transduction units/mL to avoid strong T cell media dilution.
3. Gently add 100 µL of medium containing the viral particle to each well with activated $CD3^+$ T cells. The final cultivation volume is now 200 µL.
4. Incubate cells in a humidified incubator at 5% CO_2 at 37°C.

6 Expansion: Day 3 – 6 – 8 - 10

1. On each splitting day prepare CellGenix® GMP TCM supplemented with CellGenix® IL-7 (10 ng/mL) and CellGenix® IL-15 (10 ng/mL).
2. Transfer an appropriate volume of medium to a new 96 well plate. The volumes of medium and cell suspension for the splits are listed in table on the right.
3. Pre-warm the plate to 37°C.
4. After thorough resuspension of the T cells transfer, the appropriate volume to the new 96 well plate.
5. Incubate cells in a humidified incubator at 5% CO_2 at 37°C.

Day	Ratio	Volume Medium	Volume Cells
3	1:8	175 µL	25 µL
6	1:4	150 µL	50 µL
8	1:2	100 µL	100 µL

7 Cell harvest: Day 10

1. Determine cell number, viability and transduction efficiency.

8 Additional Information

– It's important for your cultivation process to investigate the optimal seeding density for your cultivation process and system.
– The MOI directly influences your transduction efficiency as well as your process costs. Thus, the amount of lentivirus for your process should be tested in various concentrations to reach the expected results.
– From day 3 on carefully observe the density of the cells under the microscope as high densities (> 1.5×10^6 cells/mL), can result in undesired phenotypes or exhaustion. Split ratios can be adjusted accordingly.
– This 96 well plate assay is designed for media screening. If lentiviral transduction is not required increase the total seeding volume on day 0 to 200 µL, omit viral transduction on day 1 and follow the protocol as described.

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Last updated:
05 | 2023

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LM | Publication No.: SCM6021-e230501