Corning® 88-581-CM Medium for Activation and Expansion of Human T-Cells

Protocol

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T lymphocytes play an important role in cell-mediated immunity. Adoptive immunotherapy with *in vitro*-derived T cells is emerging as a powerful tool to treat cancer in a range of clinical settings. Several methods of T cell activation and expansion have been reported to use media containing various cytokines (e.g., IL-2, IL-7, and IL-21). Serum supplementation, such as human autologous plasma, provides the essential factors for cell growth and *ex vivo* expansion of T cells.

Corning 88-581-CM medium is part of the Corning 500 media series, which is widely used in adoptive immunotherapy. 88-581-CM is manufactured with high standard reagents and GMP grade raw materials. This protocol is optimized using Corning 88-581-CM medium for the activation and expansion of human T cells, using Interleukin-2 (IL-2), anti-CD3 antibody, and anti-CD28 antibody.

Content and Storage

Description	Size	Shelf Life	Qty/Pk
88-581-CM	1,000 mL	12 months	1

Reagents and Materials

- Lymphocyte Separation Medium (Corning Cat. No. 25-072-CI)
- PBS (Corning Cat. No. 21-040-CV)
- Anti-human CD3: OKT3 clone (eBioscience Cat. No. 16-0037)
- Anti-human CD28 (eBioscience Cat. No. 16-00289)
- EasySep™ Human T-Cell Isolation Kit (STEMCELL Technologies Cat. No. 17951)
- 6-well cell culture plate (Corning Cat. No. 3516)
- T-25 flask, TC-treated (Corning Cat. No. 430639)
- T-75 flask, TC-treated (Corning Cat. No. 430641)
- T-225 flask, TC-treated (Corning Cat. No. 431081)

Antibody Coating of the 6-well Plate (Day 0)

1. Prepare a 5 µg/mL solution of anti-CD3 in sterile PBS. Calculate the number of wells required for each experimental condition. To coat one well in 6-well plate, 1 mL of antibody solution is required.
2. Dispense 1 mL of the antibody solution to each well of the 6-well plate.
3. Tightly cover the plate with Parafilm™ to avoid sample evaporation, and incubate at 37°C for 2 hours or keep at 4°C overnight.
4. Just before adding cells, remove the antibody solution.
5. Rinse each well with 2 mL of sterile PBS and discard it.
6. Repeat step 5 to remove all unbound antibody from each well.

T-Cells Activation and Expansion (Day 1 to Day 14)

1. Peripheral Blood Mononuclear Cells (PBMCs) were isolated from fresh whole blood using Corning Lymphocyte Separation Medium (LSM) according to the manufacturer's protocol. Normally, ~1 x 10^6 lymphocytes can be obtained from 1 mL blood sample.
2. Inactivate the auto-plasma at 56°C for 30 minutes and then centrifuge at 800 *x g* for 20 minutes. Store the auto-plasma at 4°C for further use.
3. Human CD3+ T-cells can be magnetically sorted from the harvested PBMCs using an EasySep™ Human T-Cell Isolation Kit.

4. Resuspend the isolated CD3+ T-cells using Corning® 88-581-CM medium containing 100–200 IU/mL IL- 2 and 2% auto-plasma, with the final cell density at 1–2 x 10^6 cells/mL.
5. After rinsing the wells with PBS buffer (Step 6 in the coating procedure), add 2 mL of the cell suspension to each well, and then add anti-CD28 to cells with the final concentration at 1–2 µg/mL.
6. Incubate cells at 37°C and 5% CO_2 in a humidified incubator for 3 days.
7. From Day 4, cells will form grape-like clusters. Depending on the growth of cells, dilute the cells with fresh 88-581-CM media containing 100–200 IU/mL IL-2 and 2% auto-plasma, and keep the cell density above 0.5 x 10^6 cells/mL after each dilution.
8. Based on the cell proliferation status, transfer the cell suspension to a larger flask or gas-permeable culture bag for large-scale cell expansion. Add fresh 88-581-CM medium containing 100–200 IU/mL IL-2 and 2% auto-plasma to dilute the cell suspension every 2 to 3 days (Ensure the cell density is above 0.5 x 10^6 cells/mL).
9. Harvest T-cells around Day 14 and do quality analysis (such as cell amount, viability, and phenotype).

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

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