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

Protocol

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Corning 88-581-CM medium is part of the Corning 500 media series, which is widely used in adoptive immunotherapy. It is specifically designed for activation and expansion of cytokine induced killer (CIK) cells. 88-581-CM is manufactured with high quality reagents and GMP-grade raw materials. No other proteins are present in the medium except the injectable level of serum albumin and recombinant human insulin.

Content and Storage

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

Reagents and Materials

- Lymphocyte Separation Medium (LSM, Corning Cat. No. 25-072-CI)
- PBS (Corning Cat. No. 21-040-CV)
- INF-γ (Peprotech Cat. No. 300-02)
- IL-2 (Corning Cat. No. 354043)
- Anti-CD3 antibody (OKT3 provided by KOHJIN BIO)
- T-75 flask, TC-treated (Corning Cat. No. 430641)
- T-225 flask, TC-treated (Corning Cat. No. 431081)
- Gas-permeable cell culture bag (Corning Cat. No. 88-610-20)

CIK Cells Activation and Expansion

1. On Day 0, centrifuge the anti-coagulated blood at 400 x g for 10 minutes at room temperature (RT) and then transfer the plasma (on the top layer) into a new tube. Inactivate the auto-plasma at 56°C for 30 minutes and then centrifuge at 800 x g for 20 minutes. Store the supernatant at 4°C for further use. Add equal volume of PBS (without calcium and magnesium) as a replacement of the removed auto-plasma to maintain a constant volume and resuspend the haemocytes gently.

   Note: Pre-adding 0.1% human serum albumin (HSA) in PBS is helpful to maintain haemocytes viability (optional step).

2. Prepare peripheral blood mononuclear cells (PBMCs) from the above blood sample using LSM according to the manufacturer's directions.

   Note: Use freshly collected human blood (within 2 hours of collection) for better performance; do not use blood that is drawn more than 24 hours prior to use.

3. Wash the PBMCs once with at least 5-fold PBS buffer and then centrifuge the sample at 500 x g for 10 minutes at RT.

   Note: Pre-adding 0.1% HSA in PBS is helpful to maintain PBMCs viability (optional step).

4. Adjust the density of PBMCs to 1 to 2 x 10^6 cells/mL using 88-581-CM medium containing 1,000 IU/mL INF-γ and 5% auto-plasma (from Step 1).

   Note: Normally, more than 1 x 10^6 lymphocytes can be obtained from 1 mL blood sample.

5. Seed cells into desired culture vessel and then incubate the vessel at 37°C in a humidified atmosphere of 5% CO_2 in air.

6. After 24 hours, add IL-2 and anti-CD3 antibody at final concentrations of 500 IU/mL and 50ng/mL, respectively.
7. Around Day 5, adjust the cell density to 1 x 10^6 cells/mL by using 88-581-CM medium containing 500 IU/mL IL-2 and 5% auto-plasma.
8. Based on the cell proliferation status (typically on Day 7 before cells enter the exponential growth phase), transfer the cell suspension to a larger flask or gas-permeable culture bag for large-scale cell expansion. Use fresh 88-581-CM medium containing 500 IU/mL IL-2 and 0.5% to 1% auto-plasma to dilute the cell suspension.

   Note: Ensure that the final cell density is more than 5 x 10^5 cells/mL.
9. Depending on the cell proliferation status, replenish fresh medium containing 500 IU/mL IL-2 every 2 to 3 days; addition of 1% auto-plasma is recommended until all the auto-plasma has been exhausted.

   Note: Keep the final cell density within 1 to 2 x 10^6 cells/mL after each dilution.
10. Harvest mature CIK cells around Day 14.

For additional product or technical information, visit **www.corning.com/lifesciences** or call 800.492.1110. Outside the United States, call +1.978.442.2200 or contact your local Corning sales office.

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