1. What is Corning 88-551-CM medium?

Corning 88-551-CM medium is a serum-free medium specifically designed for activation and expansion of CIK (cytokine induced killer) and DC-CIK (dendritic cell-cytokine induced killer) cells. The medium is recommended for adoptive immunotherapy applications and in cellular immune response tests.

2. What is the packaging and shelf-life of 88-551-CM medium?

88-551-CM medium is available in a 1L size and has a shelf-life of 12 months, from date of manufacture, when stored at 2°C to 8°C.

3. Does 88-551-CM medium include IL-2?

No. Since CIK cells are dependent on exogenous cytokines such as IL-2 for activation and growth, it is necessary to add IL-2 (Corning Cat. No. 354043 or 356043) separately at a final recommended concentration of 300 IU/mL.

4. What proteins does the medium contain?

No other proteins are present in the medium except injectable grade level of human albumin and recombinant human insulin.

5. What is the manufacturing quality of 88-551-CM medium?

88-551-CM medium is produced from high quality reagents and GMP-grade raw materials and is manufactured under a robust Quality Management System. For more specific information on claims, visit the Certificates page at www.corning.com/lifesciences.

6. How do I isolate peripheral blood mononuclear cells (PBMCs) from a blood sample?

PBMCs can be isolated using Lymphocyte Separation Medium (LSM; Corning Cat. No. 25-072-CI). Typically, 1 x 10^6 PBMCs can be obtained from a 1 mL blood sample.

7. What is the recommended culture vessel for cell activation and expansion?

T-25 or T-75 tissue culture-treated flasks (Corning Cat. No. 430639 or 430641) can be used for initial cell seeding and the subsequent 14-day expansion protocol. To scale-up expansion, the cell suspension can be transferred to a larger flask (e.g., T-225; Corning Cat. No. 431080), Corning 1L cell expansion bag (Corning Cat. No. 91-200-85), or gas-permeable culture bag (Corning Cat. No. 88-610-20).

8. What is the recommended seeding density for PBMCs?

We recommend a seeding density of 1 to 2 x 10^6 cells/mL in the culture vessel. A low seeding density can lead to failure of activation of CIK cells, whilst a high seeding density can reduce activation efficiency.

9. Do I need to add auto-plasma? If so, what is the recommended percentage of supplemented auto-plasma in the medium?

88-551-CM medium is a serum-free medium. 88-551-CM medium can be used without auto-plasma to culture CIK cells; however, addition of low percentage heat inactivated auto-plasma has been demonstrated to increase activation efficiency and cell expansion. Auto-plasma can be prepared from the anti-coagulated blood sample and inactivated at 56°C for 30 minutes followed by centrifugation at 800 g for 20 minutes. We recommend adding 5% auto-plasma in the medium to activate the cells and reducing to 0.5% to 1% during the exponential growth phase (from day 7 onwards).

10. Is there any supplement that I can use to replace auto-plasma?

Yes. Human auto-plasma can be replaced by Human AB serum (Corning Cat. No. 35-060-CI).
11. What cytokines and signals are important for CIK cell activation and expansion?

CIK cells grow efficiently ex vivo following a time-sensitive protocol with the addition of INF-γ, IL-2, and OKT3 to the 88-551-CM culture medium. INF-γ, added on day 1 promotes and enhances the IL-2-mediated cellular proliferation. It also activates monocytes present in PBMCs to synergistically promote CIK cell proliferation and increase their cytotoxic activity. The recommended concentration of INF-γ is 1,000 IU/mL (Peprotech Cat. No. 300-02).

IL-2, at a final concentration of 300 IU/mL is added on day 2 and is continuously replenished throughout the 14-day culture period to induce proliferation and cytolytic activity of CIK cells. OKT3, added on day 2 at a final concentration of 50 ng/mL (Thermo Fisher Cat. No. 16-0037-81), is an anti-CD3 antibody which stimulates CIK proliferation via an IL-2 dependent mechanism.

12. What is the CIK cell culture profile using 88-551-CM medium?

CIK cell culture can be divided into two phases, activation and expansion. From day 0 to 5, cells are in their activation phase with slow growth. The cell number may be lower than the initial seeding number due to activation-induced cell death. From day 5 to 14, cells enter the exponential growth or expansion phase with a high proliferation rate. To maintain a healthy cell proliferation status, it is suggested to maintain a final cell density of 1 to 2 x 10^6 cells/mL after each replenishment of fresh media containing IL-2 and auto-plasma.

13. How long should I keep CIK cells in culture?

The standard culture period for CIK cells is 14 days with expected 100 times in expansion fold and with high viability (>90%). After 14 days, CIK cells can be harvested for downstream analysis including cell number, cellular phenotype, endotoxin, and mycoplasma testing, etc.

14. What immunophenotyping protocol is recommended to analyze the CIK cells?

Immune phenotype analysis by flow cytometry (e.g., BD Accuri™ C6) using double-positive markers CD3+/CD8+ and CD3+/CD56+ can evaluate the surface marker expression of the expanded CIK cells. Expected percentages of CD3+/CD8+ and CD3+/CD56+ cells are >60% and >5%, respectively.

Resources
- Corning 88-551-CM Lymphocyte Serum-free Medium for Activation and Expansion Culture of Human T Cells (Application Note: Corning Lit. Code: CLS-AN-443)

References

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

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