γδT Cell Culture Kit Instructions

【Product Identification】

γδT Cell Culture Kit

【Catalog Number】

MCF-020

【Packaging Specifications】

Reagent A (0.5ml/tube, 1 tube), Reagent B (0.5ml/tube, 1 tube), Reagent C (1ml/tube, 2tube), Reagent D (1.2ml/tube, 3 tube).

【Intended Use】

This kit is used for γδT cell culture. After 14 days of culture, the cell expansion fold reaches 1000 times (the total expanded cell count from 30-50 mL of blood can reach 5 to 8 billion). The proportion of γδT cells (TCRγδ+CD3+) is ≥50%, and the cell viability is >95%.

【Storage Conditions & Shelf Life】

Storage: -20°C.

Shelf Life: 12 months.

(For long-term storage, please place at -80°C)

【Sample Requirements】

This kit requires mononuclear cells separated from fresh peripheral blood or fresh umbilical cord blood, and the number of lymphocytes in the blood routine test is within the normal range. Blood collection should be sterile, and freezing should be avoided during handling and transportation. In particular, the blood collection anticoagulant must not contain EDTA and sodium citrate, and heparin anticoagulant is recommended.

【Protocol】

1. γδT cell culture flask pretreatment

Dissolve Reagent A from the kit in 20 mL of DPBS. Coat T175 culture flasks with the solution and incubate at 37°C in the dark for 2-3 hours, or at 4°C in the dark for more than 12 hours (recommended). The coated flasks are valid for 3 days.

2. Lymphocyte separation

1) Take the lymphocyte separation solution out of the 4℃ refrigerator 30 minutes in advance and place it at room temperature. Use it after the temperature rises to room temperature.

2) Centrifuge fresh heparin-anticoagulated peripheral blood from the patient at 700g for 20 minutes (with the slowest deceleration). Collect the upper plasma layer, inactivate it at 56°C for 30 minutes, then allow it to stand at 4°C for 15 minutes. Centrifuge again at 2000g for 15 minutes, and aspirate the upper plasma layer for later use.

3) To the remaining lower layer of blood, add DPBS to restore it to its original volume, and mix well. Slowly layer this mixture down the side of the tube onto an equal volume of lymphocyte separation medium, taking care not to disturb the layers. Centrifuge at 800g for 15 minutes (with slow acceleration and deceleration). After centrifugation, the liquid in the centrifuge tube will separate into four layers from top to bottom: the first layer is DPBS, the second layer is a ring-shaped milky-white layer of lymphocytes, the third layer is the transparent separation medium, and the fourth layer is red blood cells.

4) Collect the lymphocytes from the second layer into a 50 mL centrifuge tube. Add DPBS to bring the volume up to 50 mL. Centrifuge at 600g for 10 minutes, then discard the supernatant. Resuspend the cells in 50 mL of DPBS, then centrifuge again at 500g for 10 minutes, and discard the supernatant.

3. Preparation of γδT cell culture medium: Add one tube of reagent D and 1ml (40,000 IU/ml) of gentamicin to each bottle (1L) of immune cell serum-free culture medium to prepare a complete culture medium.

4. γδT cell culture

1) Aspirate the reagent in the pretreated T175 culture flask and wash the coated flask once with 10ml DPBS.

2) Resuspend the lymphocytes obtained above with 36ml γδT cell culture medium, transfer to a T175 culture flask, add 1 tube of reagent B, 80ul of reagent C and 4ml of autologous plasma, and culture in a constant temperature incubator (37℃, 5.0% CO2, saturated humidity). After thawing, the remaining reagent C is stored at 4℃ for use in the next few days.

3) On the second day, add 54ml γδT cell complete culture medium, 120ul reagent C, and 6ml inactivated plasma.

4) On the fourth day, add 45ml γδT cell complete culture medium, 100ul reagent C, and 5ml inactivated plasma.

5) On the 5th day, after the cells in the T175 culture flask are completely blown down, 500ul of reagent C and all the remaining plasma are added to the flask, the cells are transferred to a 2L cell culture bag, and γδT cell complete culture medium is added to 400ml/bag.

6) On the 7th day, the culture bag was supplemented with γδT cell complete medium to 800ml/bag.

7) On the 9th day, the culture bag was supplemented with γδT cell complete medium to 1400ml/bag.

8) On the 11th day, the culture bag was supplemented with γδT cell complete medium to 2000ml/bag, and samples were taken for bacteria, fungi, mycoplasma, and endotoxin testing.

5. γδT cell harvest

On the 14th day, γδT cells were collected by centrifugation at 2000rpm for 10mins, and then washed twice with saline at 2000rpm×8min, the cells were counted, and the cells were resuspended with saline to make the final product and packaged.

【Product Performance Specifications】

1. Appearance: The label needs to be clear and easily identifiable. There should be no liquid leakage.

2. Endotoxin: < 0.5 EU/mL.

3. Bacteria: No bacteria detected.

4. Fungi: No fungi detected.

5. Mycoplasma: No mycoplasma detected.

6. Cell growth test: After 14 days of culture, the cell expansion multiple reached 1000 times, the proportion of effective cells (CD3+CD56+ phenotype γδT cells) ≥50%, and the cell activity >95%.

【Precautions】

1. All reagents should be stored under the specified conditions to prevent microbial contamination.

2. Before use, remove the kit reagents from -20°C and allow them to slowly thaw at 4°C.

3. Before use, the kit reagents require a brief centrifugation.

4. Components from different batches of the kit must not be interchanged.

5. Expired products must not be used.

【Explanation of the logo】

None.

【References】

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[2] 康自珍,蔡海波,雷俊英,徐莉,谭文松. 体外大量扩增γδT细胞的研究[J]. 细胞与分子免疫学杂志. 2002(05).

[3] Luk PH, Negrin RS.A novel population of expanded human CD3+ CD56+ cells derived from T cells with potent in vivo antitumor activity in mice with potent severe combined immunodeficiency. J Immunol . 1994,153(4):1687-96.

【Manufacturer Information】

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