DC Cell Culture Kit Instructions

【Product Identification】

DC Cell Culture Kit

【Catalog Number】

MCF-002

【Packaging Specifications】

Reagent A (0.5ml/tube, 1 tube), Reagent B (0.5ml/tube, 1 tube), Reagent C (0.5ml/tube, 1 tube).

【Intended Use】

This cell culture kit is used for DC cell induction. After 7 days of culture, the total number of DC cells separated from 50ml of blood can reach 10 million, and the DC phenotype is $CD14^-$ $CD11c^+$>90%, $CD86^+$>90%, $CD14^+$/CD11 c^+<2%, and the cell activity is>95%.

【Storage Conditions & Shelf Life】

Storage: -20°C.

Shelf Life: 12 months.

【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. 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.

2. DC cell culture flask pretreatment

Transfer 10 mL of the non-inactivated plasma into a T75 culture flask and incubate at 37°C for 1 hour.

3. DC cell culture

1) After the culture flask coating is complete, discard the plasma in the culture flask.

2) Resuspend the collected lymphocytes using 20 mL of lymphocyte culture medium (medium containing only gentamicin). Transfer them to a T75 culture flask and incubate in a 37°C, 5% CO2 incubator for 2.5-3 hours.

3) Remove the culture flask and discard the supernatant.

4) Add 20 mL of lymphocyte culture medium (containing only gentamicin) to the flask, then add 2 mL of inactivated plasma. Add one vial of Reagent A and one vial of Reagent B. Place the flask back in the incubator for further culture.

5) On day 3, add one vial of Reagent C to the culture flask (Note: If tumor antigens are to be added, they should be added simultaneously with Reagent C). Take a sample for testing of fungi, bacteria, and mycoplasma.

4. DC Cell harvest

On day 7, collect the DC cells using trypsin digestion. Centrifuge at 1000 rpm for 5 minutes. Wash the cells twice with saline solution, then count them. Resuspend the cells in saline solution to prepare the final product, and then package it.

【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: DC cell ratio ≥ 80%, cell activity > 95%.

【Precautions】

1. After opening, store at 2~8°C.

2. The entire operation process should be carried out under sterile conditions to avoid microbial contamination.

3. If contact with the skin occurs during use, wash immediately with water. If discomfort persists, seek medical attention promptly.

4. Do not use it if the packaging shows cracks or damage.

5. Expired products must not be used.

【Explanation of the logo】

None.

【References】

Caux C, Dezutter-Dambuyant C, Schmitt D, Banchereau J. GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells. Nature. 1992,360(6401):258-61.

【Manufacturer Information】

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