NK Cell Culture Kit Instructions

【Product Identification】

NK Cell Culture Kit

【Catalog Number】

MCF-004

【Packaging Specifications】

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

【Intended Use】

This kit is used for NK cell culture. After 17 days of culture, the NK cells achieve a 1000-fold expansion (the total expanded cell count from 30-50 mL of blood can reach 5 to 8 billion). The proportion of CD3-CD56+ NK cells is ≥60%, and the cell viability 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. NK cell culture flask pretreatment

Dissolve reagent A and reagent B in 9ml DPBS in the NK cell culture kit, transfer to a T175 culture bottle, and place in a dark place at 37℃ for 2-3h, or in a dark place at 4℃ for more than 12h (recommended). The coating bottle is 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 NK cell complete culture medium

Add one tube of reagent D and 1ml (40,000 IU/ml) of gentamicin to each bottle (1L) of natural killer cell serum-free culture medium to prepare NK cell complete culture medium.

4. NK cell culture

1) Aspirate the reagent in the coated T175 culture flask and gently rinse the coated flask with 10ml DPBS. Note: When adding DPBS, do not flush the bottom of the culture flask.

2) Resuspend the lymphocytes obtained above with 17.5ml NK cell complete medium, transfer the cells to the coated T175 culture flask, add reagent C and 2ml inactivated plasma, and culture in a constant temperature incubator (37°C, 5.0% CO2, saturated humidity).

3) On the 4th day, add 36ml NK cell complete medium and 4ml inactivated plasma.

4) On the 6th day, add 74ml NK cell complete medium and 6ml inactivated plasma.

5) On the 8th day, after the cells in the T175 culture flask are completely blown down, add all the remaining plasma to the flask, transfer the cells to a 2L cell culture bag, and add NK cell complete culture medium to 400ml/bag.

6) On the 10th day, the NK cell complete medium was added to the culture bag to 800 ml/bag.

7) On the 12th day, the NK cell complete medium was added to the culture bag to 1400 ml/bag.

8) On the 14th day, the NK cell complete medium was added to the culture bag to 2000 ml/bag, and samples were taken for bacteria, fungi, mycoplasma, and endotoxin testing.

5. NK cell harvest

On the 17th day, NK cells were collected by centrifugation at 2000 rpm for 10mins, and then washed twice with saline at 2000 rpm × 8 min, 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 17 days of culture, the NK cells achieve a 1000-fold expansion, the proportion of CD3-CD56+ NK cells is ≥60%, and the cell viability is >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】

[1] R. Romee, J.W. Leong, T.A. Fehniger, Utilizing cytokines to function-enable human NK cells for the immunotherapy of cancer, Scientifica (Cairo), 2014 (2014) 205796.

[2] T. Sutlu, E. Alici, Natural killer cell-

based immunotherapy in cancer: current insights and future prospects, JIntern Med, 266 (2009) 15 4-181.

[3] M.J. Robertson, C. Cameron, S. Lazo,

K.J. Cochran, S.D. Voss, J. Ritz, Costimulation of human natural killer cell proliferation: role of a ccessory cytokines and cell contact dependent signals, Nat Immun, 15 (1996) 213-226.

【Manufacturer Information】

Manufacturer: Morecell Biomedical Technology Development Co., Ltd. Shenzhen

Address: Unit ABCDEF, 15F, Tower 6A, Baoneng Science Park, Qinghu Industrial Zone, Qingxiang Road, Longhua Street, Longhua New District, Shenzhen

Contact: +86-755-86548612