Instructions for Using NK Culture Medium KIT

I. NK Culture Medium KIT Product Portfolio:

Xeno-Free Medium for NK	Product NO.: N116-1000
NK cell Growth Factor NK	Product NO.: N116-A010
Growth Supplement	Product NO.: N116-G030
Additive C (Optimizer)	Product NO.: N116-V025

II. Culture Medium Configuration:

1) Preparation of NK cell culture medium A: Prepare 100mL NK cell serum-free culture medium. Add all additive A to the medium and add plasma at the final concentration of 2-3%. Mix well before use.

2)Preparation of NK cell culture medium B: Prepare 900mL NK cell serum-free medium. Add all additive B to the medium and add plasma at the final concentration of 2-3% for culture until the 14th or 21st day. During this period, cell counts and amplification multiples can be calculated in a timely manner based on cell's growth or needs.

III. NK Cell's Culture and Expansion

Step 1. Day 0

Collect 20-50mL umbilical cord blood (or peripheral blood) aseptically and anticoagulate with heparin (note: removal of adherent parietal cell can improve the purity of NK cell culture). Collect the plasma by low-speed centrifugation at room temperature. Place the separated human plasma at 56 ℃ for 30 minutes to inactivate. Freeze the plasma, thaw it at room temperature. Centrifuge it at 1000 rpm for 10 minutes to remove the sediment, and the plasma obtained can be used for the preparation of NK cell culture medium (note: if frozen PBMC is used for experiments, human AB type plasma needs to be prepared in advance, and the complement needs to be inactivated at 56 ℃ for 30 minutes. After that, it can be used for the preparation of NK cell serum-free culture medium (A and B fluids).

Resuspend peripheral blood cells after plasma removal with twice the amount of physiological saline. Separate the cell suspension with Ficoll lymphocyte separation solution to prepare PBMC (the collected PBMC shall be washed twice with physiological saline) and count the cells. Resuspend the cells with NK cell culture medium A (approximately 20ml) at a concentration of 1-2 × 10^6/ml and add it to the culture bottle for culture.

Step 2. Day3

Observe the cells. Microscopically, small cell colonies can be seen in the cell culture bottle. Add twice the amount of NK cell serum-free medium B solution to the bottle and continue the culture.

Step 3. Day5

Observe the cells and add NK cell serum-free medium B based on their growth status in a timely manner. Culture and expand the NK cells to the 14th or 21st day. During this period, cell counting, and expansion times can be carried out in a timely manner based on the growth statusor needs of the cells.

Step 4.

Finally, perform the cell phenotype analysis. After the experiment, collect the cells. And cells shall be labeled by fluorescent antibodies (CD3, CD8, CD56) to analyze the proportion of cells in each group.

Note: If significant decrease or stagnation of cell proliferation is found in the middle and later stages of cell expansion culture (about 10 days), culture optimization can be carried out by adding 25μl of NK cell optimization agent to every 100 ml of NK cell culture medium.

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