NK Cell Activation/Expansion Kit, human (92-01-0108)

[Components]

2 mL Anti-Biotin Microbead Particles, cell culture grade, corresponding to 4×10^8 Microbead Particles; Microbead Particles conjugated to monoclonal anti-biotin antibodies.

0.4 mL CD335 (NKp46)-Biotin, human – functional grade (100 µg/mL).

0.4 mL CD2-Biotin, human – functional grade (100 µg/mL).

[Product format] All components are supplied in azide-free buffer, Anti-Biotin Microbead Particles contain stabilizer.

[Storage] Store protected from light at 2-8 °C. Do not freeze. The expiration date is indicated on the vial labels.

[Background information]

The NK Cell Activation/Expansion Kit is designed to activate and expand human NK cells. The kit consists of Anti-Biotin Microbead Particles and biotinylated antibodies against human CD335 (NKp46) and CD2. Anti-Biotin Microbead Particles loaded with biotinylated antibodies are used to activate and expand resting NK cells purified from human blood or PBMCs.

In a first step the Anti-Biotin Microbead Particles are loaded with biotinylated antibodies. Best activation is achieved by using equal amounts of the provided biotinylated antibodies against CD335 (NKp46) and CD2.

Loaded Anti-Biotin Microbead Particles are subsequently used for the expansion of NK cells. Best activation and expansion of NK cells is accomplished by using one loaded Anti-Biotin Microbead Particle per two cells (bead-to-cell ratio 1:2). The cells are cultured for further expansion. NK cells, activated by using Anti-Biotin Microbead Particles, can be used for any downstream processing such as cytokine analysis, cytolytic activity, gene expression, or functional studies. Anti-Biotin Microbead Particles show no autofluorescence and normally do not need to be removed prior to flow cytometric analysis.

[Reagent and instrument requirements]

- Choose the appropriate MACS Separator and MACS Columns according to the number of labeled cells and to the number of total cells.
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA . Keep buffer cold (2-8 °C). Degas buffer before use, as air bubbles could block the column.
- NK Cell Isolation Kit (# 92-01-0085) or CD56 MicroBeads (# 92-01-0037).
- Medium: NK MACS Medium supplemented with 5% AB serum and 500 IU/mL human Interleukin 2 (IL-2).
- Human IL-2.
- Humidified incubator.
- Tube Rotator for loading of Microbead Particles.

[1. Loading of Anti-Biotin Microbead Particles]

▲ All steps in the protocol have to be performed under sterile conditions.

▲ Resuspend Anti-Biotin Microbead Particles thoroughly by vortexing before use, to obtain a homogenous suspension.

▲ Anti-Biotin Microbead Particles are supplied without preservative. Remove aliquots under aseptic conditions.

▲ It is recommended to load Anti-Biotin Microbead Particles in batches of 1×10^8 Anti-Biotin Microbead Particles. Loaded Anti-Biotin Microbead Particles are stable for up to 2 months when stored at 2–8 °C.

1. Pipette 100 µL of CD335 (NKp46)-Biotin and 100 µL CD2-Biotin into sealable 2 mL tube and mix well.

▲ Note: This antibody combination is optimized for achieving maximum NK cell activation and expansion.

2. Resuspend Anti-Biotin Microbead Particles thoroughly by vortexing.

3. Remove 500 µL Anti-Biotin Microbead Particles (1×10^8Anti-Biotin Microbead Particles) and add to antibody mix.

4. Add 300 µL buffer to adjust to a total volume of 1 mL.

▲ Note: Anti-Biotin Microbead Particles can be loaded in a flexible manner with biotinylated antibodies or ligands other than those supplied. If desired, add other biotinylated antibodies or ligands at appropriate concentrations and adjust with buffer to a total volume of 1 mL, accordingly.

5. Incubate for 2 hours at 2–8 °C under constant, gentle rotation by using the Tube Rotator at approximately 4 rpm (slowest permanent run program).

6. The loaded Anti-Biotin Microbead Particles (1×10^8Anti-Biotin Microbead Particles/mL) are now ready to use. **Do not remove the loaded Anti-Biotin Microbead Particles from the antibody mix.** Store at 2–8 °C for up to 2 months.

[2. Magnetic separation of NK cells using the NK Cell Isolation Kit]

▲ Isolate the NK cells according to the NK Cell Isolation Kit data sheet.

[3. NK cell activation and expansion protocol]

▲ This NK cell activation and expansion protocol is optimized for NK cells that have been purified using the NK Cell Isolation Kit, using one loaded Anti-Biotin Microbead Particle per two NK cells (bead-to-cell ratio 1:2).

▲ Volumes for activation given below are for 10^6 purified NK cells or 10^6 PBMCs. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 2×10^6 total cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend **loaded Anti-Biotin Microbead Particles** thoroughly and transfer 5 µL (5×10^5 loaded Anti-Biotin Microbead Particles) per 10^6 NK cells to a suitable tube.

▲ Note: If unloaded Microbead Particles shall be used for negative control experiments, replace loaded Anti-Biotin Microbead Particles by adding 5×10^5 of unloaded Anti-Biotin Microbead Particles per 10^6 NK cells.

2. Add 100µL culture medium to the **loaded Anti-Biotin Microbead Particles** and centrifuge at 300×g for 5 minutes.

3. Aspirate supernatant and resuspend **loaded Anti-Biotin Microbead Particles** in 50 µL of fresh culture medium.

4. Resuspend PBMCs or purified NK cells at a density of 10^6cells per 950 µL of culture medium (NK MACS Medium supplemented with 5% AB serum and 500 IU/mL IL-2).

5. Add the prepared Anti-Biotin Microbead Particles from step 3 to the 950 µL of cell suspension and mix well.

6. Add the mixture to a suitable cell culture vessel at a density of 10^6cells per mL, for example, in the wells of a 24 well cell culture plate.

7. Incubate at 37 °C and 5% CO_2.

▲ Inspect cultures daily, and add fresh medium if required.

▲ For NK cell expansion the addition of culture medium is required regularly. In the following a guideline for the stimulation and expansion of NK cells is described.

8. At day 6, gently pipette cell suspension up and down to break up clumps.

9. Determine cell number and dilute to 1–1.5×10^6 cells per mL by adding fresh culture medium (NK MACS Medium supplemented with 5% AB serum and 500 IU/mL IL-2). Transfer to a fresh culture vessel of appropriate size.

▲ NK cell expansion is donor-dependent. Best results are achieved when NK cells are maintained in a cell density of 1–1.5×10^6NK cells per mL. Depending on the expansion rate it might be necessary to split culture daily.

[4. Immunofluorescent staining]

▲ Volumes for fluorescent labeling given below are for 10^6 total cells. When working with fewer than 10^6 cells and up to 10^7 cells, use the same volumes as indicated.

▲ Microbead Particles show no autofluorescence and do not need to be removed prior to flow cytometric analysis.

▲ Scatter properties of cells may be altered due to strong interaction between cells and Microbead Particles.

1. Resuspend cells to break up cell clumps.

2. Wash cells by adding 1–2 mL of buffer per 10^6 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

3. Add 10 µL of each staining antibody, e.g., CD3-FITC and CD56-PE to 10^6 cells resuspended with buffer to a total volume of 110 µL.

4. Mix well and incubate for 10 minutes in the dark in the refrigerator (2—8 °C).

▲ Note: Working on ice requires increased incubation time. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.

5. Wash cells by adding 1–2 mL of buffer per 10^6 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

6. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.