# EasySort™ Mouse NK Cell Isolation Kit

**Cat. No: MIM005N** **Size: 10Assays/100Assays/200Assays**

| Cat. | Products | 10 Assays | 100 Assays | 200 Assays | Storage |
|---|---|---|---|---|---|
| MIM005NA | EasySort™ Mouse NK Beads Streptavidin 1.0-N | 210 μL | 700 μL × 3 | 700 μL × 6 | 2-8°C |
| MIM005NB | EasySort™ Mouse NK Cell Isolation Cocktail | 61 μL | 610 μL | 610 μL× 2 | 2-8°C |
| | Manual | | One Copy | | |

## Storage

Store at 2-8°C with shading light for 1 year. Avoid freezing and thawing.

## Detection Principle

Mouse NK cell isolation is a negative selection method to isolate NK cells from mouse spleen single cell suspensions. The principle is to use different biotinylated monoclonal antibodies to label non target cells (non-NK cells), and then remove them by streptavidin-labelled magnetic beads, so the unstimulated primitive state NK cells were isolated.

EasySort™ mouse NK cell isolation kit is a product that can isolate high purity mouse NK cells quickly and easily. The kit is suitable for isolation of NK cells from mouse spleen, and the isolated cells can be directly used for downstream applications.

## Reagents and Materials Not Supplied

1. **Reagents:**

   PBS, fetal bovine serum (FBS), EDTA

2. **Materials:**

   Disposable sterile syringe, 70 μm mesh nylon strainer, ophthalmic scissors, ophthalmic forceps, 1.5 mL/2 mL EP tube, 15 mL centrifuge tube, flow tube

3. **Instrument:**

   Optical microscope, centrifuge, 5 mL magnetic rack


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## Experimental Operation

**NOTE: The following operations must be performed under sterile conditions**

- **Isolation buffer preparation**

  Add fetal bovine serum (final concentration of 2%) and EDTA (final concentration of 2 mM) and filter the prepared buffer with 0.22 μm filtration.

  NOTE: Sealed store the prepared buffer at 4°C and use within 1 week. In addition, 2% fetal bovine serum can be replaced by 0.5% BSA.

- **Mouse spleen single cell suspension preparation**

  a) Take the fresh mouse spleen to avoid excessive connective tissue attached.

  b) Grind the spleen through a 70 μm mesh nylon strainer, rinse the cell sieve with pre-cooled PBS, and collect the cell suspension in a 15 mL centrifuge tube and centrifuge at 300×g for 5 min.

  c) Discard the supernatant, resuspend the splenocytes with isolation buffer, and filter the cells through a 70μm mesh nylon strainer, then count the cells. Adjust the cell density to $2\times10^8$ cells/mL.

  Note: Generally, about 2-4×$10^8$ splenocytes can be obtained from each mouse.

- **Cell Sorting**

  a) Prepare 50 μL of cell suspension (about $1\times10^7$ cells), add 6.1 μL Mouse NK Cell Isolation Cocktail, mix fully and incubate for 10-15 min at room temperature.

  Note: Please make sure the cells are single-cell suspension.

  b) Add isolation buffer to a final volume of 2 mL, centrifuge at 300×g for 5 min. Discard the supernatant, and then resuspend the cells with 50 μL isolation buffer.

  c) Wash Beads Streptavidin 1.0-N: Vortex beads for 20 seconds, add 20 μL Beads in 1.5 mL EP tube. Put the tube on a 5 mL magnetic rack (self-provided) and stand for 1 min. Remove the supernatant by magnetic separation, then resuspend beads with 1 mL isolation buffer, and stand for 5 minutes at room temperature. Remove the supernatant by magnetic separation.

  d) Then resuspend the Beads with 50 μL of cell suspension. Transfer the cells to the bottom of the flow tube (Note: Avoid adding along tube walls), mix fully and incubate at room temperature for 5 min.

  Note:

  ✧ If more than $1\times10^7$ cells are to be sorted, increase the amount of Mouse NK Cell Isolation Cocktail and Mouse NK Beads Streptavidin 1.0-N proportionally while ensuring the cell density remains $2\times10^8$ cells/mL. If fewer than $1\times10^7$ cells are to be sorted, resuspend the cells with 50 μL sorting buffer, add 6.1 μL Mouse NK Cell Isolation Cocktail and 21 μL washed Mouse NK Beads Streptavidin 1.0-N.

  ✧ The 5 mL flow tube is suitable for 50 μL - 1 mL cell suspension.

  e) Add isolation buffer to a final volume of 2.5 mL, mix fully with a pipette by blowing up and down for 7-8 times until no particles of magnetic beads are visible. Put the tube on a 5 mL magnetic rack (self-provided) and stand for 3 min.

  Note: Please mix the liquid thoroughly to avoid the magnetic beads clumping and affecting the isolation efficiency.

  f) Transfer the cell suspension to a clean centrifuge tube, this is the NK cells obtained from the first isolation. Add isolation buffer to a final volume of 2.5 mL into the flow tube, mix with a pipette by blowing up and down for 7-8 times until no particles of magnetic beads are visible. Put the tube on a 5


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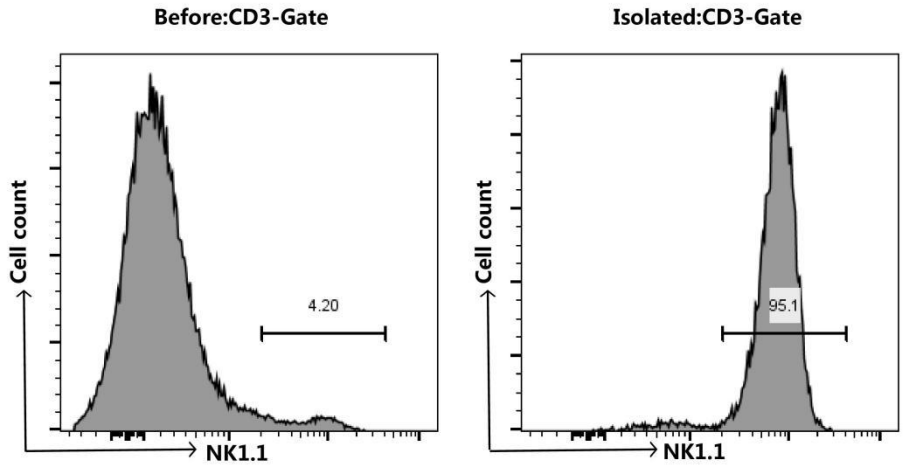
mL magnetic rack (self-provided) and stand for 3 min.

g) Transfer the cell suspension to the centrifuge tube in step e), mix the cell suspension obtained from step d) and e), centrifuge at 300×g for 5 min. Discard the supernatant, resuspend the cells with isolation buffer required for the subsequent experiments.

## Typical data




Before isolation After isolation

NK cells were isolated from the spleen cells of C57BL/6 mice, Mouse spleen cells were co-stained before and after the sorting with APC Anti-Mouse CD3 Antibody[17A2] (E-AB-F1013E) and PE Anti-Mouse CD161/NK1.1 Antibody[PK136] (E-AB-F0987D), the result shows that the proportion of CD3-NK1.1+ cells before and after the sorting of mouse spleen cells were 4.2% and 95.1%, respectively.

## Cautions

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. Avoid freezing and thawing during the use and storage of the beads.
4. The cell clusters in the cell suspension will affect the purity of cell isolation. Therefore, cell suspension should be filtered with a 70 μm mesh nylon sieve before formal isolation.
5. Cell suspension should be isolation immediately after preparation, the longer the storage time, the greater the impact on cell activity.
6. The cell suspension and magnetic beads should be added directly to the bottom of flow tube to avoid sticking to the wall, resulting in insufficient reaction and affecting the isolation efficiency.
7. In order to ensure the activity of the cells, the whole process of the experiment should be completed on ice as much as possible, except for the incubation at room temperature.
8. It is recommended to use low adsorption pipette tips and centrifuge tubes to avoid the loss of magnetic beads and antibodies due to adsorption.
9. The kit should be used in conjunction with a magnetic rack.
10. Sample type, sample preparation and experimental operation have an important impact on the final isolation cell purity.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