Human NK Cell Isolation Kit(RUO)

1 Product Information

Product Name	Model	Components
Human NK Cell Isolation Kit (RUO)	K1207-10	2 mL NK Cell Biotin-Antibody Cocktail 2 mL Streptavidin MicroBeads
Human NK Cell Isolation Kit (RUO,Trial)	K1207-10T	200 μL NK Cell Biotin-Antibody Cocktail 200 μL Streptavidin MicroBeads

2 Product description

The Human NK Cell Isolation Kit is used for quick and easy separation of NK cells from single-cell suspensions of human peripheral blood mononuclear cells (PBMC), tumor tissue, thymus, lymph nodes or other tissues.

Principle: Non-NK cells are removed by adding an appropriate amount of antibodies and MicroBeads to the single-cell suspension to obtain the target NK cells. Negatively selected NK cells can be directly used for downstream experiments such as cell culture and flow cytometry etc.

3 Capacity

For 10^9 total cells, up to 100 tests (10^7 cells/test).

4 Transportation and storage

Shipping at 2 ~ 8°C;

Store protected from light at 2 ~ 8°C. Do not freeze. This kit is valid for 12 months.

5 Requirements for reagents and instruments

Buffer: phosphate buffered saline (PBS) pH 7.2, containing 0.5% bovine serum albumin (BSA) and 2 mM EDTA

LarSep Columns (RWD, model: HCSC-25)

40 μm cell filter

Note:

- PBS containing Ca^{2+} or Mg^{2+} is not recommended.
- To prevent air bubbles from blocking the column, please avoid using buffers that contain many bubbles.

6 Method for use

6.1 Sample preparation

(1) For human peripheral blood, it is suggested to use density gradient centrifugation to obtain peripheral blood mononuclear cells (PBMC); for other tissues, prepare single-cell suspension with the single-cell suspension dissociator or manually.

(2) Rinse the 30 μm cell filter with buffers, and then filter the cell suspension with the filter. After preparation, store the cell suspension at 2 ~ 8°C.

(3) (Optional) Dead cells or erythrocytes may affect the separation, which can be depleted by The Dead Cell Removal Kit and Red Blood Cell Lysis Buffer.

6.2 Magnetic labeling

Note:

- The reagents given in the following steps can process 10^7 cells. If there are less than 10^7 cells, add the reagents according to 10^7 cells; if there are more than 10^7 cells, increase the reagents accordingly in proportion.
- Operate as quickly as possible, keep cells cold, and use pre-cooled solutions to reduce nonspecific cell labeling.

(1) Count the cell number and adjust the cell concentration to 1×10^8 cells/mL.

(2) Take 100 μL cell suspension (containing 10^7 cells).

(3) Add 20 μL NK Cell Biotin-Antibody Cocktail.

(4) Mix well and incubate at 2 ~ 8 ℃ for 5 min.
(5) Add 20 μL Streptavidin MicroBeads.
(6) Mix well and incubate at 2 ~ 8°C for 10 min.
(7) Add 1 ~ 2 mL buffer and mix well.
(8) Perform magnetic separation. (Filter the sample before separation if the cell concentration is too high or there are too many cell clumps and aggregates).

6.3 Magnetic separation

Note: Before adding buffer in the following steps, make sure that all the buffer added to the column in the previous step drains away (i.e. no continuous droplets are dripping from the lower port of the column).

(1) Put the column in a suitable magnetic field.
(2) Wash the column with 4 mL buffer.
(3) Add the cell suspension to the column.
(4) Collect the effluent. 2 ~ 3 mL buffer is added to continue collecting effluents to enrich NK cells (containing unlabeled NK cells).
(5) When buffer added in the previous step drains away, remove the column from the magnetic field and replace the collection tube with a new one.
(6) Add 1 ~ 2 mL buffer into the column, and then flush out the buffer with the plunger supplied with the column to obtain the magnetically labeled non-NK cells.

7 Precautions

(1) This product is for research use only.
(2) Do not mix and match components from different lots or different kits.
(3) This kit is valid for 12 months, and RWD does not guarantee the validity of expired products.
(4) All operations should be performed under sterilized conditions.
(5) Cells should be incubated at 2 ~ 8°C. High temperatures or extended incubation durations may result in non-specific labeling.

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