Human NK Cell Enrichment Kit

Cat No: KD102800EK

Description:

Isolate untouched and highly purified natural killer (NK) cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or lysed leukapheresis samples by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 95% purity
- Untouched, viable cells

This kit targets non-NK cells for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and magnetic particles, and separated without columns using a magnet. Desired cells are simply poured off into a new tube. Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction.

Kit Components:

1. Human NK Cell Enrichment Cocktail – 1 x 1ml
2. D Magnetic Particles – 2 x 1ml

Storage:

Store the components at 2 - 8°C. Do not freeze.

Sample Preparation:

Prepare a PBMC suspension from whole peripheral blood by centrifugation over a density gradient medium. If using previously frozen PBMCs, incubate the cells with DNase I Solution at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes prior to labeling and separation. Filter aggregated suspensions through a 37 µm cell strainer for optimal results. After preparation, resuspend cells at 5 x 10^7 cells/mL in recommended medium.

LEUKAPHERESIS

1. Add an equal volume of Ammonium Chloride Solution to the leukapheresis sample.

NOTE: If working with large volumes (> 150 mL), concentrate leukapheresis sample first by centrifuging at 500 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original leukapheresis volume with recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of

recommended medium). For small volumes (≤ 150 mL), add Ammonium Chloride Solution directly to the cell suspension.

2. Incubate on ice for 15 minutes.

3. Centrifuge at 500 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.

4. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 150 x g for 10 minutes at room temperature with the brake off.

Carefully remove the supernatant.

5. Repeat step 4 one or more times until most of the platelets have been removed (indicated by a clear supernatant).

6. Resuspend the cells at 5 x 10^7 cells/mL in recommended medium.

Recommended Medium:

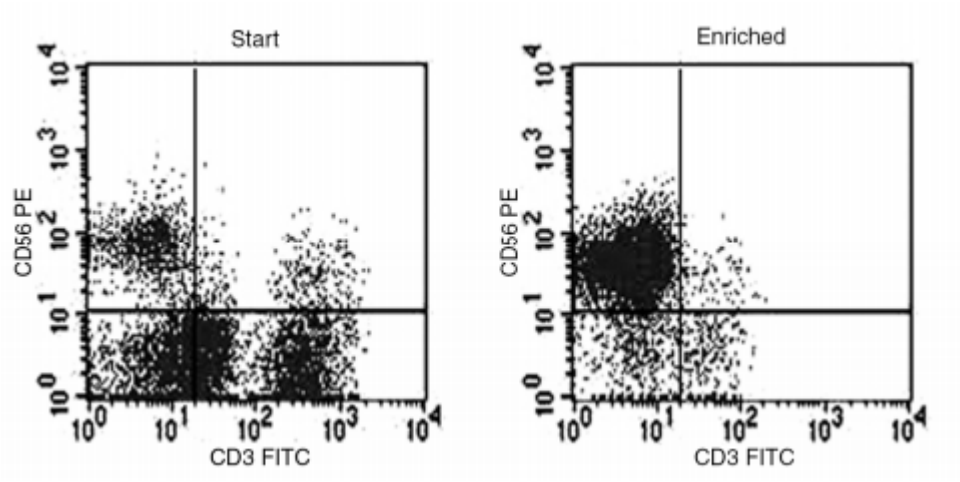
Cell separation buffer (PBS containing 2% fetal bovine serum (FBS) and 1 mM EDTA). Medium should be free of Ca++ and Mg++.

Procedure:

1. Prepare sample at the 5 x 10^7 cells/mL cell concentration within 0.25 - 2 mL volume range.
2. Add 5ml sample to polystyrene round-bottom tube
3. Add Enrichment Cocktail to 50 µL/mL of sample.
4. Mix and Incubate at room temperature for 10 minutes
5. Vortex Magnetic Particles NOTE: Particles should appear evenly dispersed.
6. Add Magnetic Particles to 100 µL/mL of sample.
7. Mix and incubate at room temperature for 5 minutes
8. Add recommended medium to top up the sample to 2.5ml. Mix by gently pipetting up and down 2 3 times.
9. Place the tube (without lid) into the magnet and incubate room temperature for 2.5 minutes
10. Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the enriched cell suspension into a new tube.
11. Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring off the enriched cell suspension into a new tube. Use a new 5 mL tube
12. Remove the tube from the magnet and add recommended medium to top up to 2.5 mL. Mix by gently pipetting up and down 5 - 6 times.
13. Place the tube (without lid) into the magnet and incubate.

14. Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring off the enriched cell suspension into a new tube. Combine with poured-off fraction from step 6 Isolated cells are ready for use

Data:



Starting with previously frozen mononuclear cells containing more than 10% NK cells, the NK cell content of the enriched fraction typically ranges from 73 - 95%. In the above example, the purities of the start and final enriched fractions are 10% and 96%, respectively.

NOTE: The NK cell content (CD56+CD3-) of the enriched fraction varies, depending on the starting sample. Purities may be lower when starting with samples containing less than 10% NK cells.