KBM NK kit

Basic Manual

Kohjin Bio Co., Ltd.

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1．Product Description

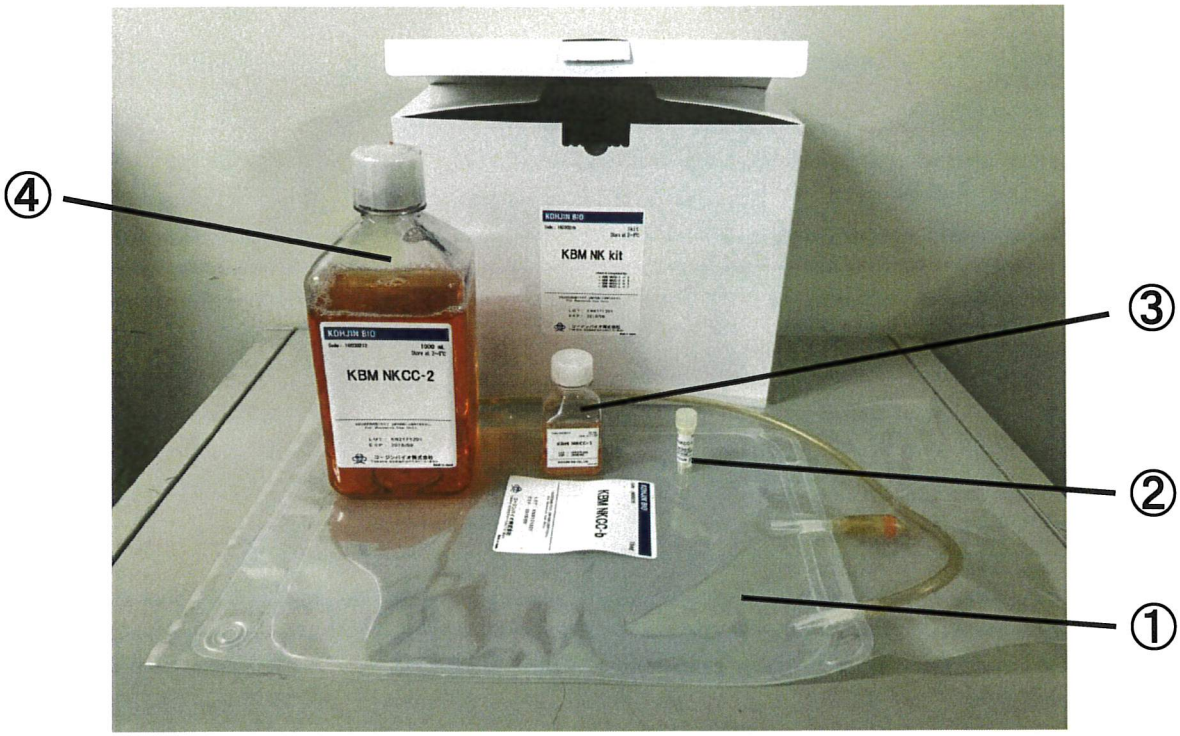
1－1．Brief Introduction

KBM NK kit is designed to proliferate NK cells selectively from human peripheral blood lymphocytes.

In our actual cultivation tests, it is found that the cell numbers grow up to 3 billion ~ 3.6 billion and more than 50% of that become NK cells in many tests. (※1)

1－2．Kit Components

	Product Name	Purpose of use
①	NKCC- b	Culture bag
②	NKCC- c	Coating agent
③	NKCC-1	Initial culture medium
④	NKCC-2	Expansion culture medium



(※1) There are rare cases NK ratio isn't shown over 50% according to each individual.

2. Protocol

2-1. Coating Method

Preparation

- NKCC- c
- 15mL or 50mL tube
- 75cm^{2}Flask

Method

1) Add NKCC-c 1mL to 13mLD-PBS(-) (Photo 1)

2) Pour fully into the 75cm^{2}Flask with 14mL coating agent. (Photo 2)

3) Leave the coated flask for a day under refrigeration (2-8℃), or for 4 hours at a temperature of 37℃

4) Remove the coating agent, and rinse it twice with D-PBS(-)

5) Keep refrigerated (2-8℃) until used (Use enable for several day if it isn't dry)

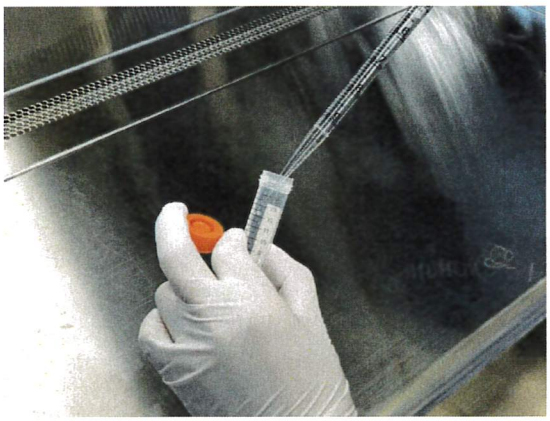


Photo 1

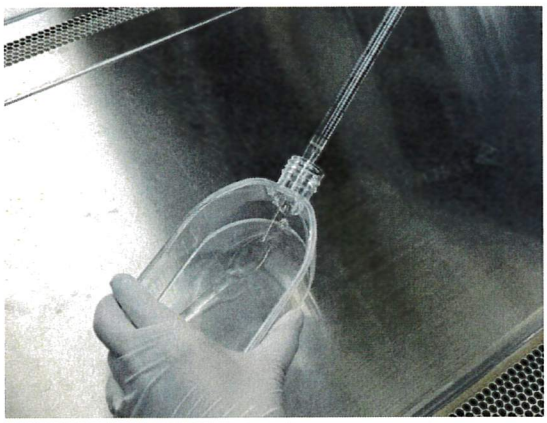


Photo 2

2．Protocol

2-2. Cultivation Method

Preparation

・Human peripheral blood (30 to 50 mL) PBMC collection use (using heparin tube)

・Human peripheral blood (10 to 20 mL) Autologous serum collection use (using plain tube)

・NKCC-1

・NKCC-2

・NKCC- b

・IL-2（Concentration about 500,000IU/mL）

1）Isolate PBMC from human peripheral blood (30 mL to 50 mL) by a density gradient centrifugation method (using Ficoll, etc) . At this time, the blood plasma of the supernatant in the centrifuge tube is stored separately for cultivation.

2）Count only nuclear cell count, not red blood cell, with staining solution such as Türk's solution.

3）Add 10% of serum to NKCC-1 and the cells will be seeded at a cell density of 400,000 cells / cm^2 and a cell concentration of 100,000 μg / mL (about 30 million cells in case of a 75cm^2 flask).

4）Leave it standing at a 37℃ in a 5%CO_2 Incubator for 4~6 days.

5）4~6days later, when the culture medium change into yellow as Photo 3 and observe the colony formation, count the cell numbers. If the cell numbers have grown 2 to 3 times, transfer the cell suspension to a centrifuge tube, and centrifuge at 1200 rpm for 5 minutes. Then remove the supernatant and collect the cells.

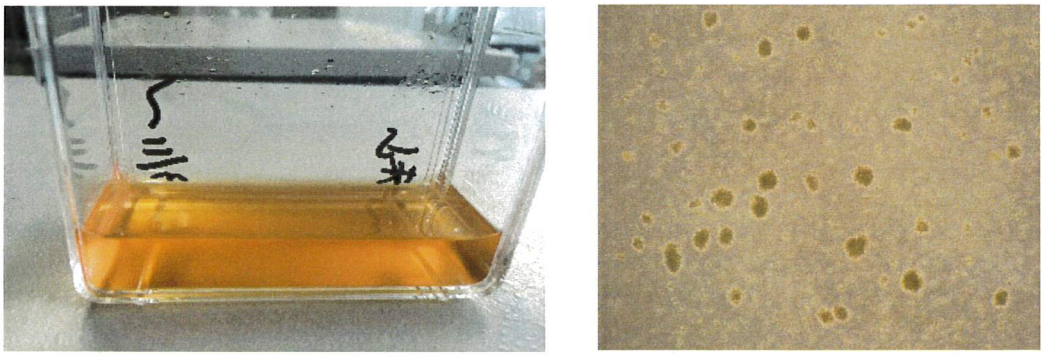


Photo 3　Color change （Left）　Colony formation（Right）

2．Protocol

2-2. Cultivation Method （Con't)

6）Connect the NKCC-b with uncapped port to a syringe of about 50 mL, and re-suspend the collected cells in NKCC-2, then pour the solution of culture and cell suspension into a syringe. (Photo 4)

At this time, the grading of cell concentration is 500,000 cells / mL, and add IL-2 in NKCC-2 to be 1000IU/mL for use.

Besides, add whole amount of inactivated Plasma which is collected from PBMC isolation.

7）Add culture medium to be about 250,000 cells/mL every few days to avoid exceeding 2 million cells/mL. After 10-14 days cultivation, collect cells when the cell concentration became 2-3 million cells/mL.

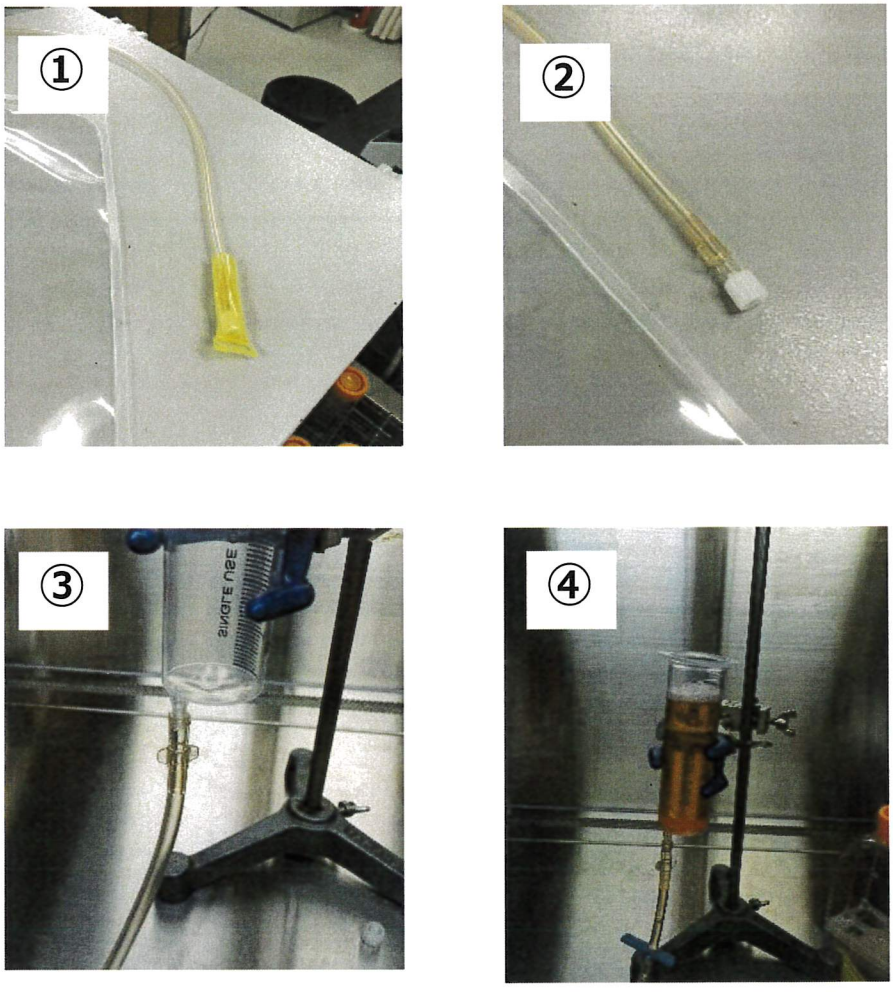


Photo 4　Method of culture filling to NKCC- b
① Capped syringe port
② Uncapped syringe port
③ Connection NKCC- b to a syringe
④ Culture filling

3. Example of NK cell culture

Culturing condition

Cell : PBMC
Initial culture medium : NKCC-1＋10% human serum
Expansion culture medium : NKCC-2＋human plasma(Inactivation)＋1000IU/mL IL-2
Flask for Initial culture medium : T75Flask(Corning 430641U)coated with NKCC- c
Culture bag : NKCC- b

1. Seeded PBMC in coated T75Flask (NKCC-1 20mL adding serum 2mL) and cultured in a 5%CO^2 incubator at 37℃.
2. After 5 days cultivation, collected cells when the number of cells became 8.2×10^7 (1200rpm×5min).
3. Added 200mL of NKCC-2 to be the cell concentration of 400,000cells/mL. At that time, added 10mL of inactivated human serum which was collected from PBMC isolation.
4. After 7 days, the culture medium changed into yellow, then added the remaining 800mL of NKCC-2 into the bag.
5. After 10 days, counted the cell numbers and checked cell surface markers by flow cytometer and measured Cytotoxic activity with K562.

Table1 Changes in cell number

Days of culture	Cell yield	Viability
0	2.0×10^7	—
5	8.2×10^7	—
1 0	3.7×10^9	94.6%

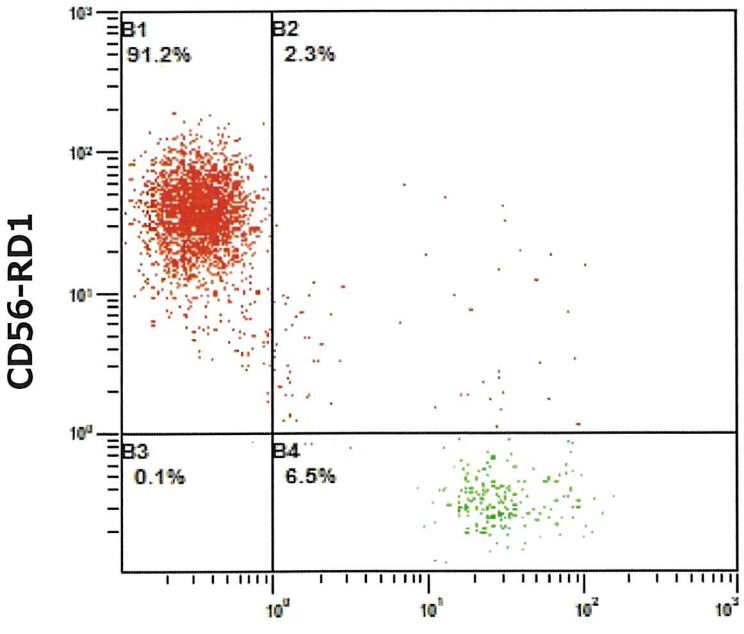


Fig.1 Surface makers by flow cytometer

Table2 Percentage of Cytotoxicity

E/T	Cytotoxicity 2 hr	4hr
12	100%	100%
24	100%	100%

Table3 Surface makers by flow cytometer

Marker	Rate
CD56+/CD3-	91.2%
CD56+/CD3+	2.3%
CD56-/CD3+	6.5%
CD56-/CD3-	0.1%

4．Contact

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