

KBM NK Kit

Applications

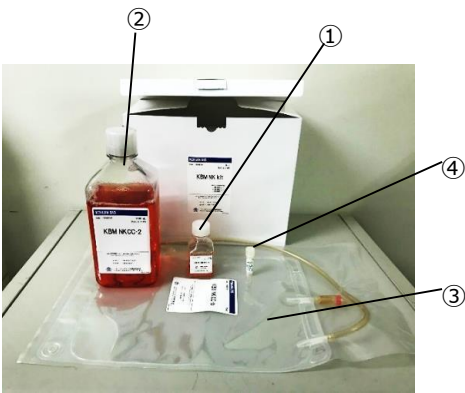
KBM NK kit is selectively cultivates NK cells from PBMC (Peripheral Blood Mononuclear Cells) derived from human peripheral blood efficiently and easily.

Characteristics

In 10 days of culture, it is able to proliferate cells by 100 to 150 times (Up to 3.6 billion cells at maximum), and more than 50 % of them to be NK cells (*).
 * Rarely, the NK ratio does not exceed 50% due to individual differences.

Cell Culture Example

1. PBMC 2.0×10^7 cells were seeded into T75 flasks pre-coated (NKCC-1 20 mL, serum 2 mL added) and incubated in a 5 % CO2 incubator at 37 °C.
2. At 5 days of culture, the cells were collected the number of cells reaches to 8.2×10^7 cells. Transfer cells for expansion of culture (1200 rpm x 5 min).
3. Add 200 mL of NKCC-2 so that the cell concentration gets 4.0×10^5 cells/mL. Add inactivated (56 °C, 30 min) human serum (10 mL) obtained at the time of PBMC separation.
4. At 7 days of culture, add the remaining 800 mL of NKCC-2 to the bag, after it was confirmed that the medium had turn to yellow.
5. At 10 days of culture, count the number of cells. Confirm the cell surface markers (CD56, CD3) and measure the cytotoxic activity using K 562 (target cancer cell) on a flow cytometer.



The kit is composed by;
 ①KBM NKCC -1 (Medium for primary culture)
 ②KBM NKCC -2 (Medium for expansion)
 ③KBM NKCC-b (culture bag)
 ④KBM NKCC-c (coating agent)

【Culture Conditions】

Cultured Cells	PBMC(Peripheral Blood Mononuclear Cells)
Primary Medium	NKCC-1+10% Human Serum (inactivated)
Expansion Medium	NKCC-2+Human Serum(inactivated)
Culture Vessel	T75 Flask(Corning,430641U) Coated with NKCC-c
Culture Bag	NKCC-b
Primary Culture	PBMC 2.0×10^7 cells/NKCC-1 20 mL, Serum 2ml
Expansion Culture	4.0×10^5 cells/NKCC-2 200 mL, Plasma 10 mL collected during PBMC isolation
Culture Environment	Culture under static condition at 37 °C• 5%CO2

Table1 Changes in cell number

Days of Culture	Cell yield	Viability
0	2.0×10^7	-
5	8.2×10^7	-
10	3.7×10^8	94.6%

Table2 Percentage of Cytotoxicity

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

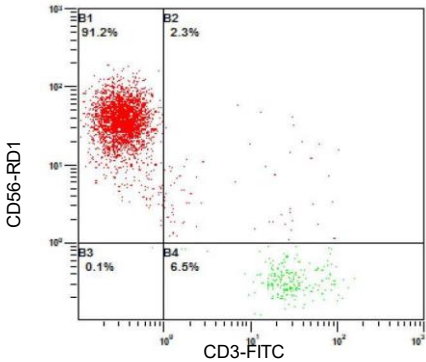


Fig.1 Surface makers by flow cytometer

Product No	Product Name	Size	Price	Shelf Life	Storage
16030210	KBM NK Kit	1 Kit	JPY 57,500	9 months	2-8 °C

* This product is sold for research purposes only.