



# BINKIT<sup>®</sup> for NK cells expansion from PBMCs

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Revised at 2014/03/04

## Product features

- Natural killer (NK) cells can be expanded from human peripheral blood mononuclear cells (PBMCs) without using feeder cells.
- NK cells can be expanded several hundred to several thousand-fold by 2 - 3 weeks of culture.
- One kit is sufficient to expand NK cells from 20 - 50 ml of whole blood.

Kit name	Catalog No.	Amount
BINKIT <sup>®</sup>	N501-1	1 kit
	N501-2	2 kits
	N501-4	4 kits
	N501-8	8 kits

Kit components	Catalog No.	Amount
One kit of BINKIT <sup>®</sup> includes:		
NK Cell Initial Flask (M)	N104	1 flask (75 cm <sup>2</sup> )
NK Cell Initial Medium	N115a	45 ml
NK Cell Initial Cocktail	N115b	1.9 ml
NK Cell Subculture Medium	N201	1000 ml

## Intended use

For research use only.

Not for use in diagnostic procedures.

## Storage

Store at 2 - 8 °C. Protect from light.

## Shelf life

Three months after production or until expiration date.

## Other supplies required

Ficoll-Paque (GE Healthcare, Sweden)

Sterile PBS

FBS or autologous plasma (It is desirable to be heat-inactivated at 56 °C for 30 minutes.)



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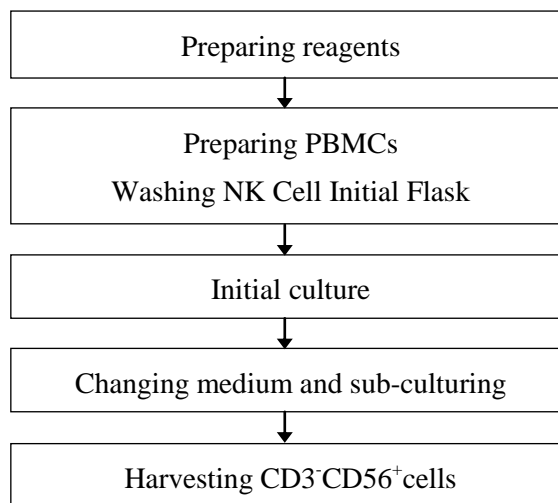
Sterile conical centrifuge tubes

Sterile culture flasks

## Precautions

NK Cell Initial Flask may carry condensation on the surface, which does not adversely affect the performance of the kit.

## Procedure overview



## Procedures

### Preparing reagents

NK Cell Initial Medium and NK Cell Subculture Medium should be supplemented with 5 % (v/v) of heat-inactivated FBS or autologous plasma.

### Preparing peripheral blood mononuclear cells (PBMCs)

Isolate PBMCs from whole human blood by density gradient centrifugation using Ficoll-Paque.

### Washing NK Cell Initial Flasks

Add 10 ml PBS to an NK Cell Initial Flask. Slant the flask to cover the entire surface with PBS. Aspirate the liquid completely from the flask. Care should be taken so as not to scratch the surface of the flask. Repeat the washing process two more times.





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### **Culturing NK cells from PBMCs**

Suspend the PBMCs in NK Cell Initial Medium at  $1 \times 10^6$  cells/ml. Add 40  $\mu$ l of NK Cell Initial Cocktail to 1 mL of the cell suspension. Transfer the cell suspension to the pre-washed NK Cell Initial Flask. Incubate under 5 % CO<sub>2</sub> at 37 °C for 3 days.

### **Changing medium and sub-culturing**

Transfer floating as well as adherent cells to a conical centrifuge tube and centrifuge at 200 x g for 8 minutes. Remove the supernatant and re-suspended the cells at  $1 \times 10^6$  cells/ml in NK Cell Subculture Medium supplemented with 5 % (v/v) of heat-inactivated FBS or autologous plasma. The cell suspension is transferred to conventional culture flasks and is cultured under 5 % CO<sub>2</sub> at 37 °C. Cells should be sub-cultured every 2 - 3 days by suspending cells in completed NK Cell Subculture Medium at  $0.8 \times 10^6$  cells/ml.

### **Suggested culturing period**

2 - 3 weeks.

### **Effects**

CD3<sup>-</sup>CD56<sup>+</sup> NK cells will be expanded several hundred to several thousand-fold in 2 - 3 weeks of culture, making more than 50 % of cultured cells to be CD3<sup>-</sup>CD56<sup>+</sup> NK cells.

### **References**

Xuwen Deng, Hiroshi Terunuma, Mie Nieda, Weihua Xiao, Andrew Nicol, Synergistic cytotoxicity of ex vivo expanded natural killer cells in combination with monoclonal antibody drugs against cancer cells, *Int Immunopharmacol* **14** (2012) 593-605

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