BINKIT® for NK cells expansion from PBMCs

Revised at 2013/08/23

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®	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® includes:		
NK Cell Initial Flask (75 cm^2)	N104 (M)	1 flask
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 producing or until the 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.)

Sterile conical centrifuge tubes

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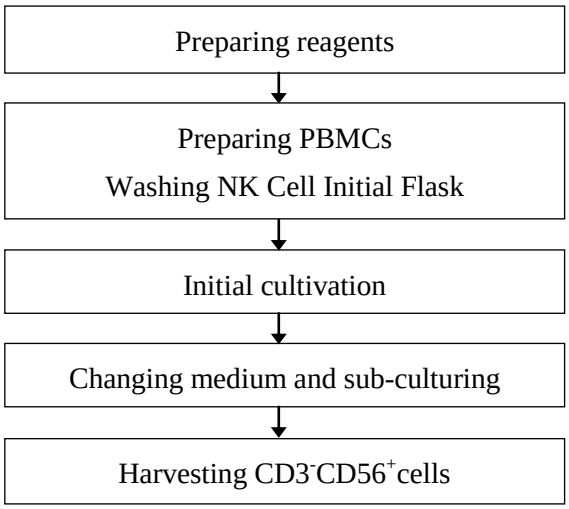
Sterile culture flasks

Precautions

NK Cell Initial Flask may carry condensations on the surface, which do 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 not to scratch the surface of the flask. Repeat the washing process two more times.

Culturing NK cells from PBMCs

Suspend the PBMCs in NK Cell Initial Medium at 1×10^6 cells/ml. Add 40 µl of NK Cell Initial Cocktail to the cell suspension. Transfer the cell suspension to the pre-washed NK Cell Initial Flask. Incubate under 5 % CO_2 at 37 °C for 3 days.

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Changing medium and sub-culturing

Transfer floating as well as adherent cells to a conical centrifuge tube and centrifuged at 200 x g for 8 minutes. Remove the supernatant and re-suspended the cells at 1×10^6 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 cultured under 5 % CO_2 at 37 °C. Cells should be sub-cultured every 2 - 3 days by suspending cells in completed NK Cell Subculture Medium at 0.8×10^6 cells/ml.

Suggested culturing period

2 - 3 weeks.

Effects

$CD3^-CD56^+$ NK cells will be expanded several hundred to several thousand-fold in 2 - 3 weeks of culture to make more than 50 % of cultured cells to be $CD3^-CD56^+$ NK cells.

References

Xuewen 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

Xuewen Deng, Mie Nieda, Hiroshi Terunuma, Ex vivo expanded natural killer cells can possibly kill cancer stem cells, *18th ISCT Annual Meeting* 2012

Xuewen Deng, Hiroshi Terunuma, Mie Nieda, Cytotoxicity of expanded NK cells against cancer cells is enhanced by monoclonal antibody drugs, *19th ISCT Annual Meeting* 2013

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