BINKIT®-NK for NK cells expansion from PBMCs

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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®-NK	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®-NK includes:		
NK Cell Initial Flask (M)	N104	1 flask (75 cm^2)
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 - 10 °C. Protect from light.

Shelf life

One year 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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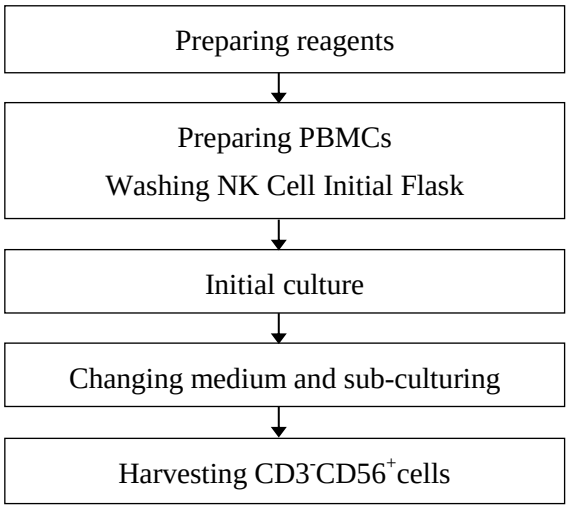
Sterile conical centrifuge tubes

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 1×10^6 cells/ml. Add 40 µl 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_2 at 37 °C for 3 days.
(A culture Flask should be kept with the culture surface down.)

Changing medium on Day3

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 1×10^6 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_2 at 37 °C.

Sub-culturing

Cells should be sub-cultured every 2 - 3 days by adding freshly NK Cell Subculture Medium between a density of 0.8×10^6 cells/ml and 3.0×10^6 cells/ml. The maximum density should not be more 3.0×10^6 cells/ml. The density of adding freshly NK Cell Subculture Medium is recommended between 0.8×10^6 cells/ml and 1.2×10^6 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, making more than 50 % of cultured cells to be $CD3^-CD56^+$ NK cells.

References

Deng X, Terunuma H, Nieda M, Xiao W, Nicol A: Synergistic cytotoxicity of ex vivo expanded natural killer cells in combination with monoclonal antibody drugs against cancer cells. Int Immunopharmacol 14: 593-605, 2012

Terunuma H, Deng X, Nishino N, Watanabe K: NK cell-based autologous immune enhancement therapy (AIET) for cancer. Stem Cells Regenerative Med 9: 9-13, 2013

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Deng X, Terunuma H, Terunuma A, Takane T, Nieda M: Ex vivo-expanded natural killer cells kill cancer cells more effectively than ex vivo-expanded gd T cells or ab T cells. Int Immunopharmacol 22: 486-491, 2014

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