**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**
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BINKIT® | N501-1 | 1 kit
 | N501-2 | 2 kits
 | N501-4 | 4 kits
 | N501-8 | 8 kits

**Kit components**

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

![Diagram](image)

Preparation
- Preparing reagents
- Preparing PBMCs
- Washing NK Cell Initial Flask
- Initial cultivation
- Changing medium and sub-culturing
- Harvesting CD3 CD56+ cells

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% CO₂ at 37 °C for 3 days.
**BINKIT® for NK cells expansion from PBMCs**

**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 x 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% CO₂ at 37 °C. Cells should be sub-cultured every 2 - 3 days by suspending cells in completed NK Cell Subculture Medium at 0.8 x 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

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