BINKIT® for NK cells expansion from PBMCs

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
- BINKIT®

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>N501-1</td>
<td>1 kit</td>
</tr>
<tr>
<td>N501-2</td>
<td>2 kits</td>
</tr>
<tr>
<td>N501-4</td>
<td>4 kits</td>
</tr>
<tr>
<td>N501-8</td>
<td>8 kits</td>
</tr>
</tbody>
</table>

Kit components
- One kit of BINKIT® includes:
  - NK Cell Initial Flask (M) N104 1 flask (75 cm²)
  - 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.)
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

<table>
<thead>
<tr>
<th>Preparing reagents</th>
<th>Preparing PBMCs</th>
<th>Washing NK Cell Initial Flask</th>
<th>Initial culture</th>
<th>Changing medium and sub-culturing</th>
<th>Harvesting CD3/CD56+ cells</th>
</tr>
</thead>
</table>

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.
Culturing NK cells from PBMCs
Suspend the PBMCs in NK Cell Initial Medium at $1 \times 10^6$ 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 % CO₂ 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 % CO₂ at 37 °C. Cells should be sub-cultured every 2 - 3 days by suspending cells in completed NK Cell Subculture Medium at $0.8 \times 10^6$ 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
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*
BINKIT® for NK cells expansion from PBMCs

MANUFACTURED BY:
Biotherapy Institute of Japan Ltd.
2-4-8 Edagawa, Koto-ku, Tokyo, 135-0051 Japan
Phone: +81-3-5632-6080
FAX: +81-3-5632-6083
HP: http://www.bij-net.com
E-mail: info@bij-net.com