

# Feeder-free Expansion of Human NK cells in G-Rex® 6M Well Plate

## OTHER MATERIALS REQUIRED

- Recombinant Human IL-2 (R&D Systems, Catalog # BT-002-AFL)
- Recombinant Human IL-12 (R&D Systems, Catalog # 219-IL)
- Recombinant Human IL-15 (R&D Systems, Catalog # BT-015)
- Recombinant Human IL-18 (R&D Systems, Catalog # 9124-IL)
- Recombinant Human IL-21 (R&D Systems, Catalog # 8879-IL)
- Pipettes and pipette tips
- 15 mL and 50 mL Polypropylene Centrifuge Tubes
- G-Rex® 6M Well Plate (Wilson Wolf, Catalog #80660M)
- 37 °C, 5% CO<sub>2</sub> incubator
- Inverted microscope
- Flow cytometer
- Cell counting materials
- Centrifuge
- Human AB Serum (optional)

## RECOMMENDED PROTOCOL

1. Isolate PBMCs using desired protocol and enrich NK cells using commercially available kits. Frozen stocks of NK cells may be used after thawing.
2. Pre-warm the required amount of ExCellerate™ Human NK Cell Expansion Medium to room temperature. Supplement pre-warmed medium with the following cytokines at the following concentrations: Recombinant Human IL-2 (250 IU/mL), Recombinant Human IL-12 (10 ng/mL), Recombinant Human IL-15 (10 ng/mL), Recombinant Human IL-18 (10 ng/mL), and Recombinant Human IL-21 (10 ng/mL). The required amount of medium may vary depending on the G-Rex® culture vessel used. For example, the maximum working volume of a single well in a G-Rex® 6M well plate is 100 mL.  
**Note:** Human serum may be added at 2 - 5%.
3. Determine the cell density of enriched NK cells. Dilute the cell suspension to desired cell density in cytokine-containing medium. The recommended initial seeding number of NK cells in a G-Rex® culture vessel is  $2.5 \times 10^6$  per cm<sup>2</sup>.
4. Seed NK cells.
5. Incubate at 37 °C and 5% CO<sub>2</sub> in a humidified incubator for 5 days. It is recommended not to disrupt cell clumps during this time.
6. To refresh the culture medium at day 5, prepare the required amount of medium containing following cytokines: Recombinant Human IL-2 (500 IU/mL), Recombinant Human IL-15 (20 ng/mL), Recombinant Human IL-18 (20 ng/mL), and Recombinant Human IL-21 (20 ng/mL). Replace half of the culture medium with pre-warmed cytokine-containing medium. After adding the medium, resuspend cells carefully.  
**Note 1:** Human serum may be added at 2 - 5%.  
**Note 2:** It is recommended not to add IL-12 cytokine from day 5.  
**Note 3:** If the larger vessels are used, add cytokine-containing medium maintaining the following concentrations of the following cytokines: Recombinant Human IL-2 (250 IU/mL), Recombinant Human IL-15 (10 ng/mL), Recombinant Human IL-18 (10 ng/mL), and Recombinant Human IL-21 (10 ng/mL).
7. Refresh the culture medium every 3-4 days by repeating Step 6.
8. Cultured NK cells may be reseeded in new wells. The recommended reseeding number of NK cells is  $5 \times 10^4$  per cm<sup>2</sup> in G-Rex® culture vessel.