

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

OTHER MATERIALS REQUIRED

- Recombinant Human IL-2 (R&D Systems, Catalog # BT-002-AFL)
- Pipettes and pipette tips
- 15 mL and 50 mL Polypropylene Centrifuge Tubes
- G-Rex® 6M Well Plate (Wilson Wolf, Catalog #80660M)
- 37 °C, 5% CO₂ incubator
- Inverted microscope
- Flow cytometer
- Cell counting materials
- Centrifuge
- Human AB Serum (optional)

RECOMMENDED PROTOCOL

1. Prepare γ -irradiated feeder cells. The recommended dosage of irradiation is 100 Gy.
2. Isolate PBMCs using desired protocol and enrich NK cells using commercially available kits. Frozen stocks of NK cells and feeder cells may be used after thawing.
3. Pre-warm the required amount of ExCellerate™ Human NK Cell Expansion Medium to room temperature. Supplement pre-warmed medium with Recombinant Human IL-2 cytokine at 50 IU/mL. The required amount of medium may vary depending on G-Rex® culture vessels. 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%.
4. Determine the cell density of enriched NK cells and feeder cells. Dilute the cell suspension to the desired cell density in cytokine-containing medium. The recommended seeding number of NK cells in a G-Rex® culture vessel is 5×10^4 - 5×10^5 per cm² for feeder cell-based stimulation.
5. Seed NK cells and feeder cells at 1:2 ratio (NK cell: feeder cell). The ratio may be optimized.
6. Incubate at 37 °C and 5% CO₂ in a humidified incubator for 5 days. It is recommended not to disrupt cell clumps during this time.
7. Refresh half of the culture medium at day 5 with fresh medium containing IL-2 cytokine at 100 IU/mL. After adding the medium, resuspend cells carefully. The final working concentration of IL-2 cytokine will be 50 IU/mL.
Note 1: Human serum may be added at 2 – 5%.
Note 2: If larger vessels are used, add pre-warmed cytokine-containing medium maintaining 50 IU/mL of IL-2 cytokine concentration.
8. Refresh the culture medium every 3-4 days by repeating Step 7.
9. Restimulation with irradiated feeder cells is recommended every week for optimal expansion. For restimulation, cultured NK cells may be reseeded in new wells. The reseeded number of NK cells can be 5×10^4 - 5×10^5 per cm² in a G-Rex® culture vessel. The recommended NK cell: feeder cell ratio is 1:1.