

# NK-92 Cell Line Culture Protocol

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## Purpose

This protocol describes thawing, routine culture, IL-2-supplemented maintenance, passaging, and cryopreservation of the **human NK-92 cell line**. NK-92 is a suspension NK-like cell line requiring continuous IL-2 supplementation for viability, proliferation, and maintenance of cytotoxic phenotype.

## Critical notes (read before starting)

- NK-92 is a **human clinical-grade NK cell line** and must be handled under strict BSL2 conditions.
- NK-92 requires **continuous IL-2 supplementation** for survival. Never culture NK-92 in IL-2-free medium.
- NK-92 is typically cultured at **200–500 IU/mL IL-2** (lab-validated range should be consistent).
- NK-92 grows in suspension but forms **loose aggregates**; avoid over-pipetting or harsh centrifugation.
- Passage NK-92 before cultures become dense or acidic; overgrown cultures lose cytotoxicity and viability.
- Cryopreserved NK-92 must be thawed quickly and washed immediately to remove DMSO.

### Warning

NK-92 viability rapidly decreases if IL-2 is omitted or if medium becomes depleted. Always add IL-2 fresh to pre-warmed medium before feeding or splitting.

### Tip

For reproducibility, record IL-2 batch number, concentration added, split ratio, and passage number at every passage.

## **Approximate timing**

- Thawing: 15–20 min
- Routine maintenance and splitting: 10–20 min
- Cryopreservation: 20–30 min
- Hands-on time per culture session: ~15–30 min

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## Procedure

### Step 1 – Medium and reagent preparation

1. Prepare complete NK-92 medium:
  - RPMI 1640
  - 10% heat-inactivated FCS
  - 1% Pen/Strep
2. Prepare IL-2 working aliquots.
3. Supplement complete medium with IL-2 immediately before use:
  - **200 IU/mL** final (or your lab-validated concentration).
4. Pre-warm:
  - NK-92 complete medium + IL-2
  - PBS (if washing is needed) to **37 °C**.

#### Note

IL-2 is unstable when stored in complete medium. Always add fresh, immediately before feeding.

### Step 2 – Thawing NK-92 cells

1. Remove a cryovial from liquid nitrogen and place immediately in a **37 °C water bath**.
2. Gently swirl until only a small ice crystal remains.
3. Disinfect the vial and transfer to a biosafety cabinet.
4. Transfer the contents to a 15 mL tube containing **9–10 mL** pre-warmed medium **without IL-2** to dilute DMSO.
5. Centrifuge at **300 g for 5 min**.
6. Carefully aspirate supernatant.
7. Gently resuspend the pellet in **10–15 mL** NK-92 complete medium **with IL-2**.
8. Transfer cells to a T25 or T75 flask and incubate at **37 °C, 5% CO<sub>2</sub>**.

#### Warning

Do not expose thawed cells to DMSO for more than a few minutes; wash immediately after thawing.

### Step 3 – Routine culture and passaging

#### Recommended density

- Maintain cultures between **2 × 10<sup>5</sup>** and **1 × 10<sup>6</sup>** cells/mL.

## Procedure

1. Gently pipette or swirl flask to resuspend cells uniformly.
2. Remove an aliquot for counting and viability.
3. If density exceeds **1 × 10 cells/mL**, split culture:
  - Remove part of the culture (e.g.,  $\frac{1}{2}$ – $\frac{3}{4}$  volume).
  - Replace with fresh NK-92 medium + IL-2.
4. Restore cultures to **2–3 × 10 cells/mL**.
5. Return flasks to incubator.

## Full medium change for stressed cultures

1. Collect cells and centrifuge at **300 g for 5 min**.
2. Aspirate depleted medium.
3. Resuspend cells in fresh complete NK-92 medium + IL-2.

### Note

Fresh IL-2 medium is essential after any decline in viability; NK-92 responds rapidly to improved culture conditions.

## Step 4 – Preparing NK-92 cells for experiments

1. For functional assays, activate cultures 24 hours before experiments using:
  - Fresh NK-92 complete medium + IL-2
2. Seed cultures at defined densities (e.g., **2–3 × 10 cells/mL**).
3. Avoid using NK-92 beyond a set passage number (e.g., **<20 passages** post-thaw).

## Step 5 – Cryopreservation of NK-92 cells

### Freezing medium

- **90% FCS + 10% DMSO**, or
- Mix cell suspension 1:1 with **BUF-GEN-FREEZE-MIX-001** for a final 10% DMSO freezing medium.

## Procedure

1. Harvest NK-92 cells during exponential growth.
2. Centrifuge at **300 g for 5 min**.
3. Resuspend cells at **5–10 × 10 cells/mL** in freezing medium.
4. Aliquot **1 mL** per labelled cryovial.
5. Place into a **controlled-rate freezing container** (Mr Frosty).

6. Freeze at  $-70/-80\text{ }^{\circ}\text{C}$  for 12 hours.
7. Transfer to liquid nitrogen for long-term storage.

## Materials

### Reagents

Reagent	Supplier	Cat. #	Notes
RPMI 1640	Various	—	Base medium
Fetal calf serum (FCS)	Various	—	10% in culture, 90% in freezing medium
Penicillin/Streptomycin	Various	—	1% final in medium
Recombinant human IL-2	PeproTech/etc.	—	200 IU/mL final
DMSO, cell-grade	Sigma/etc.	—	10% final for freezing
PBS, 1×	Various	—	For optional washes

### Disposables

Item	Specification
T25 / T75 flasks	Vent-cap, sterile
15 mL / 50 mL tubes	Sterile
Cryovials	LN compatible
Serological pipettes	Sterile (5–25 mL)
Pipette tips	Sterile (10–1000 $\mu\text{L}$ )

### Equipment

Equipment	Specification / Notes
CO incubator	37 $^{\circ}\text{C}$ , 5% CO
Biological safety cabinet	BSL2 work
Benchtop centrifuge	300 g for washing
Water bath	37 $^{\circ}\text{C}$ for thawing
Controlled-rate freezer	e.g., Mr Frosty
$-70/-80\text{ }^{\circ}\text{C}$ freezer	For initial freezing
Liquid nitrogen storage	Long-term storage
Cell counter/hemocytometer	For counting

### Buffers used

- **BUF-GEN-FREEZE-MIX-001** – 2× freezing medium (for final 10% DMSO)
- PBS (laboratory standard)

## Troubleshooting

Issue	Possible cause	Suggested solution
Poor viability after thaw	Slow thawing; prolonged DMSO exposure	Thaw rapidly; wash promptly; ensure gentle handling
Slow growth	Insufficient IL-2; old medium	Increase IL-2 (within validated range); refresh medium
Excessive clumping	Overgrowth or nutrient depletion	Split earlier; replace medium entirely
Reduced cytotoxicity	High passage number; inconsistent IL-2	Use low-passage cells; standardise IL-2 concentration
Contamination	Aseptic technique failure	Discard culture and restart from frozen stock

## Safety (brief)

- NK-92 is a human-derived cell line and must be handled under **BSL2**.
- Wear gloves, lab coat, and eye protection.
- Dispose of all culture waste as biohazardous.
- Handle DMSO cautiously; it increases dermal absorption of contaminants.
- Follow institutional protocols for cryogen handling and LN<sub>2</sub> storage.

## Version history

Version	Date	Author	Changes
v1.0	2025-11-21	Dillon Corvino	Initial Quarto protocol for NK-92 culture