

NK-92 Cell Line Culture Protocol

Abdullah Lab, IMMEI, University Hospital Bonn

Protocol ID: HUM-NK92-CULTURE-001

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Author: Dillon Corvino

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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% CO₂**.

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 × 10⁵** and **1 × 10⁶** 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 μL) |

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 storage.

Version history

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