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** .

> **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** and **1 × 10 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., ½–¾ 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-FREEZE-MIX` 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 °C** for 12 hours.

7. Transfer to liquid nitrogen for long-term storage.

Buffers Required

Full composition, preparation, storage, and safety information for each buffer lives in its own linked buffer document — only a concise per-buffer summary is reproduced here, generated automatically from that document.

2x Freezing Medium

Component	Final concentration / amount
RPMI 1640 (R0)	base medium (7 mL per 10 mL batch)
Fetal calf serum	10% (v/v)
DMSO (cell-grade)	20% (v/v)

- **Final volume:** 10 mL (2x concentrate; example batch, scale as needed)
- **Preparation note:** Mix gently by inversion; do not vortex. Keep on ice until use.
- **Storage:** Use fresh on day of preparation; do not store; discard any unused buffer.
- **Caveat:** DMSO is toxic at room temperature - keep the 2x mix on ice and mix with cells immediately after preparation. Final working freezing medium (after 1:1 mix with cells) contains 10% DMSO.

Full composition, preparation steps, storage, and safety information: `BUF-FREEZE-MIX`.

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 °C, 5% CO
Biological safety cabinet	BSL2 work
Benchtop centrifuge	300 g for washing
Water bath	37 °C for thawing
Controlled-rate freezer	e.g., Mr Frosty
−70/−80 °C freezer	For initial freezing
Liquid nitrogen storage	Long-term storage
Cell counter/hemocytometer	For counting

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

Issue	Possible cause	Suggested solution
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
v1.1	2026-09-18	Dillon Corvino	Replaced `# Buffers used` (bare `library(tidyverse)`, leaked R package-startup warnings) with an auto-generated `# Buffers Required` section; moved it from after Materials to before Materials. Fixed stale buffer-ID reference `BUF-GEN-FREEZE-MIX-001` -> `BUF-FREEZE-MIX` in Step 5