KHYG-1 Cell Line Culture Protocol

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Purpose

This protocol describes the thawing, routine culture, maintenance, passaging, and cryopreservation of the human KHYG-1 NK-like cell line. KHYG-1 cells grow as a suspension culture and require IL-2 supplementation for optimal growth and function.

Critical notes (read before starting)

- KHYG-1 is a **human NK-like suspension cell line**; treat all material as BSL2.
- Maintain cultures in **logarithmic growth phase**; avoid overgrowth and nutrient depletion.
- IL-2 is required for optimal growth and should be **added fresh** to complete medium at each medium change.
- Always pre-warm media and PBS to **37 °C** before use.
- Avoid excessive centrifugation speeds and harsh pipetting to prevent cell damage.
- Use controlled-rate freezing and appropriate freezing medium for cryopreservation.

> **Warning**
>
> Failure to supplement medium with IL-2 or allowing cultures to overgrow can lead to reduced viability, altered phenotype, and inconsistent experimental results.

> **Tip**
>
> Keep a simple culture log listing passage number, split ratio, IL-2 batch and concentration, and any deviations. This improves reproducibility across experiments.

Approximate timing

- Thawing: 15–20 min
- Routine split/medium change: 10–20 min
- Cryopreservation: 20–30 min

Total hands-on time per session: ~15–30 min.

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Procedure

Step 1 – Medium and reagent preparation

1. Prepare **KHYG-1 complete medium** based on R10 (BUF-R10-MED):
   - RPMI 1640
   - 10% heat-inactivated FCS
   - 1% Pen/Strep
2. Prepare **IL-2 stock** according to manufacturer's instructions.
3. Immediately before use, supplement complete medium with IL-2:
   - Final concentration: **100–200 IU/mL** (use a lab-validated concentration consistently).
4. Pre-warm:
   - KHYG-1 complete medium + IL-2
   - PBS (if washing is needed)

   to **37 °C**.

> **Note**
>
> IL-2 should not be stored long-term in medium; add fresh to pre-warmed R10 immediately before feeding or splitting cultures.

Step 2 – Thawing KHYG-1 cells

1. Remove a cryovial of KHYG-1 cells from liquid nitrogen and immediately place it in a **37 °C water bath**.
2. Gently swirl the vial until only a small ice crystal remains (1–2 min).
3. Disinfect the outside of the vial with 70% ethanol and transfer to a biosafety cabinet.
4. Transfer the vial contents to a 15 mL tube containing **9–10 mL** pre-warmed KHYG-1 complete medium **without IL-2** to dilute DMSO.
5. Centrifuge at **300 g for 5 min** at room temperature.
6. Carefully aspirate the supernatant without disturbing the cell pellet.
7. Resuspend the cell pellet gently in **10–15 mL** pre-warmed KHYG-1 complete medium **with IL-2**.
8. Transfer cells to an appropriate flask (e.g. T25 or T75).
9. Place the flask in a **37 °C, 5% CO incubator**.

> **Warning**
>
> Do not leave cells in freezing medium (DMSO-containing) after thawing. Wash promptly to minimise DMSO toxicity.

Step 3 – Routine culture and passaging

Recommended density range

- Maintain cultures between approximately **2 × 10 and 1 × 10 cells/mL**.

Monitoring and splitting

1. Gently resuspend the culture by pipetting up and down or gently swirling the flask.
2. Remove a small aliquot for counting and viability assessment.
3. If the cell density exceeds **1 × 10 cells/mL**, split the culture:
    - Remove a defined volume of culture (e.g. half to three-quarters of the flask volume).
    - Replace with an equal volume of **fresh pre-warmed KHYG-1 medium with IL-2**.
    - This corresponds to approximately a **1:2–1:4 split**, depending on growth.
4. Aim to re-establish cultures at **2–3 × 10 cells/mL** after splitting.
5. Return flasks to the incubator (37 °C, 5% CO).

Medium change for lower-density cultures

- If cells are at acceptable density but medium appears acidic or exhausted:
    1. Gently resuspend cells.
    2. Transfer to a 15 mL tube and centrifuge at **300 g for 5 min**.
    3. Aspirate supernatant and resuspend in fresh medium with IL-2 at the desired density.
    4. Return to flask and incubator.

> **Note**
>
> Maintain consistent IL-2 concentrations at each passage and medium change. Record IL-2 lot numbers for traceability.

Step 4 – Short-term maintenance and experimental setup

1. For experiments, seed KHYG-1 cells at a defined density consistent with experimental requirements (e.g. **2–3 × 10 cells/mL**).
2. Use fresh medium with IL-2 on the day before and the day of the experiment to ensure cells are in active growth phase.
3. Avoid using cultures older than a defined passage number (e.g. **>20–25 passages** post-thaw) for critical experiments; restart from frozen stock as needed.

Step 5 – Cryopreservation of KHYG-1 cells

Freezing medium

- Standard freezing medium for KHYG-1:
  - **90% FCS + 10% DMSO**
    or
  - Cell suspension in R10 mixed 1:1 with **BUF-FREEZE-MIX** (2× freezing medium) to yield final 10% DMSO.

Freezing procedure

1. Ensure KHYG-1 cells are in exponential growth phase (healthy, non-overgrown cultures).
2. Transfer the culture to a 15 or 50 mL tube and centrifuge at **300 g for 5 min**.
3. Aspirate the supernatant and gently resuspend the pellet in freezing medium at **5–10 × 10 cells/mL**.
4. Aliquot **1 mL** of cell suspension per labelled cryovial (include cell line name, passage number, date, and your initials).
5. Place vials into a **controlled-rate freezing container** (e.g. Mr Frosty).
6. Freeze at **−70/−80 °C** for at least **12–24 hours**.
7. Transfer vials to **liquid nitrogen** for long-term storage.

> ⚠ Warning
>
> Avoid extended handling of cells at room temperature in freezing medium; DMSO is toxic and should be minimised outside of the controlled freezing process.

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.

R10 Medium (RPMI + 10% FCS)

Component	Final concentration / amount
RPMI 1640	to 500 mL
Fetal calf serum (FCS)	10% (v/v)
Penicillin/Streptomycin	1% (v/v)

- **Final volume:** 500 mL
- **Preparation note:** Gently invert to mix; do not vortex.
- **Storage:** 4C, protected from light; use within 4 weeks; do not freeze.

Full composition, preparation steps, storage, and safety information: `BUF-R10-MED`.

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 for KHYG-1 medium
Fetal calf serum (FCS)	Various	—	10% v/v in complete medium; 90% in freezing medium
Penicillin/Streptomycin	Various	—	1% v/v in complete medium
Recombinant human IL-2	PeproTech/others	—	Added fresh to 100–200 IU/mL
DMSO, cell culture grade	Sigma/others	—	10% final for freezing
PBS, 1×	Various	—	For optional washes

Disposables

Item	Specification
T25 / T75 tissue culture flasks	Vent-cap, sterile
15 mL and 50 mL tubes	Sterile
Cryovials	Suitable for liquid nitrogen
Serological pipettes	Sterile (5–25 mL)
Pipette tips	Sterile (10 µL–1000 µL)

Equipment

Equipment	Specification / Notes
CO incubator	37 °C, 5% CO
Biological safety cabinet	Class II, BSL2 work
Benchtop centrifuge	For 15/50 mL tubes, 300 g
Controlled-rate freezing unit	e.g. Mr Frosty
−70/−80 °C freezer	For initial freezing
Liquid nitrogen storage	For long-term storage
Water bath	37 °C, for thawing
Cell counter/hemocytometer	For cell counting and viability

Troubleshooting

Problem	Possible cause	Suggested solution
Slow growth or low expansion	Insufficient IL-2; suboptimal passage density	Verify IL-2 concentration; maintain cells in log phase; refresh medium
Drop in viability after thaw	Prolonged DMSO exposure; slow thaw	Thaw rapidly; wash DMSO promptly; ensure gentle handling

Problem	Possible cause	Suggested solution
Culture appears granular or clumpy	Overgrowth; depleted medium	Split earlier; replace medium completely; re-seed at lower density
Contamination (bacterial/fungal)	Aseptic technique failure	Discard contaminated culture; disinfect incubator if needed; restart from stock
Variable functional readouts	Passage-dependent changes; inconsistent IL-2	Use defined passage range; standardise IL-2 concentration and medium changes

Safety (brief)

- Handle KHYG-1 cells as human-derived BSL2 material.
- Work in a Class II biological safety cabinet with appropriate PPE (lab coat, gloves, eye protection).
- Dispose of all cell culture waste (media, flasks, pipettes) as biohazardous material.
- Handle DMSO with care; avoid skin contact and inhalation.
- Follow institutional guidelines for cryogen handling and storage in liquid nitrogen.

Version history

Version	Date	Author	Changes
v1.0	2025-11-21	Dillon Corvino	Initial Quarto protocol for KHYG-1 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