

# 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-GEN-R10-MED-001):
  - 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  $2 \times 10$  and  $1 \times 10$  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  $1 \times 10$  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  $2\text{--}3 \times 10$  cells/mL after splitting.
5. Return flasks to the incubator (37 °C, 5% CO<sub>2</sub>).

### 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.

#### **i** 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.  $2\text{--}3 \times 10$  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-GEN-FREEZE-MIX-001** (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.

## 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

## Buffers used

- **BUF-GEN-R10-MED-001** – R10 complete medium (RPMI + 10% FCS + 1% Pen/Strep).
- **BUF-GEN-FREEZE-MIX-001** – 2× freezing medium (optional, when preparing final 10% DMSO freezing medium).
- PBS (standard laboratory PBS).

## 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
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.