K-562 Cell Line Culture Protocol

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Purpose

This protocol describes the thawing, routine culture, maintenance, passaging, and cryopreservation of the human K-562 erythroleukemia cell line. K-562 cells grow in suspension and require regular monitoring of density to maintain viability and consistent growth rates.

Critical notes (read before starting)

- K-562 cells grow as **suspension cultures** and should remain in exponential growth phase.
- Do not allow cultures to exceed recommended density, as nutrient depletion reduces viability and alters phenotype.
- K-562 cells are of human origin—treat all cultures and waste as BSL2.
- Always pre-warm media and reagents to **37 °C** before use.
- Ensure gentle handling; harsh centrifugation or over-pipetting can damage suspension cells.
- Cryopreservation requires **10% DMSO**, and cells should be frozen using a controlled-rate freezing container.

> **Warning**
>
> Overgrowth (tight clumps, very dense cultures) leads to poor viability and altered functional responses. Split cultures **before** they exceed recommended density.

> **Tip**
>
> Record passage number and split ratios at every passage. This improves reproducibility when using K-562 cells as targets or stimulator cells.

Approximate timing

- Thawing: 15–20 min
- Routine medium change / split: 10–15 min
- Cryopreservation: 20–30 min
- Total hands-on time per session: ~15–30 min

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Procedure

Step 1 – Reagents and medium preparation

1. Pre-warm complete culture medium:
    - RPMI 1640
    - 10% FCS
    - 1% Pen/Strep
2. Pre-warm PBS (if washing is needed).
3. Label flasks with:
    - Cell line name (K-562)
    - Passage number
    - Date
    - Operator initials

Step 2 – Thawing K-562 cells

1. Pre-warm **10–15 mL** of complete medium to 37 °C.
2. Thaw a cryovial of K-562 cells in a 37 °C water bath:
    - Gently swirl and remove when only a small ice crystal remains.
3. Transfer contents into a 15 mL tube containing **10 mL** pre-warmed complete medium.
4. Centrifuge at **300 g for 5 min** at room temperature.
5. Remove supernatant and gently resuspend the pellet in **10–15 mL** fresh complete medium.
6. Transfer cells to a T25 or T75 flask and incubate at **37 °C, 5% CO** .

> **Note**
>
> The centrifugation step removes DMSO, preventing toxicity during recovery.

Step 3 – Routine culture and passaging

Recommended density

- Maintain between $\mathbf{2 \times 10}$ and $\mathbf{1 \times 10}$ **cells/mL**.

Procedure

1. Gently resuspend the culture by pipetting up and down.
2. Count cells using trypan blue or an automated counter.
3. If density $> 1 \times 10$ cells/mL:
   - Split culture **1:2 to 1:5** depending on growth.
4. Add fresh, pre-warmed complete medium to adjust culture to:
   - **2–3 $\times$ 10 cells/mL**
5. Return to incubator (37 °C, 5% CO).

> ⚠ Warning
>
> Do not allow cultures to sit for more than 3–4 days without splitting. Nutrient depletion leads to apoptosis and growth arrest.

Step 4 – Short-term storage / holding conditions

- Cultures may be held at **2–4 $\times$ 10 cells/mL** for 24–48 h without splitting.
- If cultures appear slow or viability drops:
  - Increase medium volume
  - Spin down and resuspend in entirely fresh medium

Step 5 – Cryopreservation of K-562 cells

Preparation of freezing medium

- **90% FCS + 10% DMSO**
  (or use BUF-FREEZE-MIX diluted 1:1 with cell suspension)

Freezing procedure

1. Collect cells in exponential growth phase.
2. Centrifuge at **300 g for 5 min**.
3. Resuspend in freezing medium at **1–10 $\times$ 10 cells/mL**.
4. Aliquot **1 mL** into labelled cryovials.
5. Place vials into a **controlled-rate freezing container** (e.g. Mr Frosty).
6. Freeze at **−70/−80 °C overnight**.
7. Transfer vials 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% v/v in medium
Penicillin/Streptomycin	Various	—	1% v/v final
DMSO (cell culture grade)	Sigma/others	—	10% final in freezing medium
PBS, 1×	Various	—	For washing (optional)

Disposables

Item	Specification
T25 / T75 flasks	Vent-cap, sterile
15 mL / 50 mL tubes	Sterile
Cryovials	LN2-compatible
Pipette tips	Sterile (10 µL – 1000 µL)
Serological pipettes	Sterile (5–25 mL)

Equipment

Equipment	Specification / Notes
CO incubator	37 °C, 5% CO
Benchtop centrifuge	300 g for washing
Biological safety cabinet	BSL2 handling
Water bath	37 °C for thawing
Cell counter/hemocytometer	For counting
Controlled-rate freezer	e.g. Mr Frosty
Liquid nitrogen storage	Long-term cryostorage

Troubleshooting

Issue	Possible cause	Solution
Low viability after thaw	Slow thawing or DMSO exposure	Thaw rapidly; wash immediately after thaw
Clumping	High density or depleted medium	Dilute, split earlier, or replace medium entirely
Slow growth	Old medium, poor serum batch	Prepare fresh medium; test new batch of FCS

Issue	Possible cause	Solution
Sudden drop in viability	Contamination or overgrowth	Check culture; discard and restart from stock

Safety (brief)

- Handle all human-derived cell lines using BSL2 precautions.
- Wear gloves, lab coat, and eye protection.
- Dispose of liquid and solid waste as biohazardous material.
- DMSO increases skin permeability—avoid contact.

Version history

Version	Date	Author	Changes
v1.0	2025-11-21	Dillon Corvino	Initial Quarto protocol for K-562 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