NK Cell Culture Medium (cRPMI for Murine NK Cells)

Abdullah Lab, IMMEI, University Hospital Bonn

2025-11-21

Protocol ID: BUF-MUS-NK-MED-001
Version: v1.0
Author: Dillon Corvino

Purpose

This medium is used for in vitro culture and proliferation assays of murine NK cells. It is based on complete RPMI 1640 supplemented with nutrients essential for lymphocyte viability and proliferation. **IL-15 is added fresh immediately before plating cells** and is not included in the stock medium.

Working buffer composition

Component	Final concentration	Notes
RPMI 1640	—	Base medium
Fetal calf serum (FCS)	10% (v/v)	Heat-inactivated
Penicillin/Streptomycin	1% (v/v)	100× stock → 1× final
HEPES	10 mM (1% v/v)	Buffering at 37 °C
Sodium pyruvate (NaPy)	1 mM (1% v/v)	Energy substrate
Non-essential amino acids (NEAA)	1% (v/v)	Supports lymphocyte metabolism
-mercaptoethanol (-ME)	50 M (e.g. 0.05 mM)	Reduces oxidative stress

> **Note**
>
> IL-15 is **not included** in this stock medium. It must be added to a final concentration of 100 IU/mL immediately before culturing NK cells.

Preparation

Stock reagents required

Reagent	Stock concentration	Notes
RPMI 1640	—	Sterile-filtered
FCS, heat-inactivated	—	10% final
Pen/Strep	100×	1% final
HEPES buffer	1 M	10 mM final (1% v/v)
Sodium pyruvate (NaPy)	100 mM	1 mM final (1% v/v)
NEAA	100×	1% final
-mercaptoethanol	50 mM or 14.3 M	See dilution below

Preparation of 500 mL NK medium (cRPMI base)

1. In a sterile bottle, combine:
   - **450 mL** RPMI 1640
   - **50 mL** FCS (10% final)
2. Add supplements:
   - **5 mL** Pen/Strep (100× stock → 1% final)
   - **5 mL** HEPES (1 M stock → 10 mM final)
   - **5 mL** Sodium pyruvate (100 mM stock → 1 mM final)
   - **5 mL** NEAA (100× stock → 1% final)
3. Add -mercaptoethanol:
   - Final concentration: **50 M**
   - If using 50 mM stock: add **0.5 mL**
   - If using 14.3 M stock: prepare a working dilution before adding
4. Mix gently by inversion; do **not vortex**.
5. Label with:
   - "NK medium (cRPMI base)"
   - Date of preparation
   - Expiry (4–5 weeks at 4 °C)

> ⚠ Warning
>
> -mercaptoethanol is hazardous. Handle it in a fume hood and avoid skin contact. Do not open concentrated -ME bottles inside a biosafety cabinet.

Usage

- Warm medium to **37 °C** before adding cells.
- **Add IL-15 fresh** immediately prior to plating NK cells:
  - Final concentration **100 IU/mL**.
- Do not store IL-15 in the base medium.

Storage and stability

- Store at **4 °C**, protected from light.
- Use within **4–5 weeks** of preparation.
- Do **not freeze**.

Reagent details

Reagent	Supplier	Cat. #	Notes
RPMI 1640	Various	—	Base medium
FCS	Various	—	Heat-inactivated
Pen/Strep	Various	—	100× working stock
HEPES	Various	—	1 M stock
Sodium pyruvate	Various	—	100 mM stock
NEAA	Various	—	100× stock
-mercaptoethanol	Sigma/etc	—	Handle with caution
IL-15 (added fresh)	PeproTech/etc	—	Not included in base medium

Safety

- Handle -mercaptoethanol and all supplements using gloves and eye protection.
- -ME requires fume hood handling due to volatility.

- Prepare medium under aseptic conditions inside a biosafety cabinet.
- Dispose of expired or contaminated medium as biohazardous waste.

Version history

Version	Date	Author	Changes
v1.0	2025-11-21	Dillon Corvino	Initial Quarto buffer document for NK medium