

# ACK Red Blood Cell Lysis Buffer

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**Protocol ID:** BUF-ACK-LYSIS-001

**Version:** v1.0

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## Purpose

ACK lysis buffer is used to selectively lyse erythrocytes in single-cell suspensions derived from mouse or human tissues (e.g. liver digests, spleen, PBMC preparations). The reagent maintains leukocyte integrity while efficiently removing red blood cells during brief room-temperature incubation.

## Working buffer composition

Component	Final concentration	Notes
Ammonium chloride (NH <sub>4</sub> Cl)	150 mM	Primary erythrocyte-lysing component
Potassium bicarbonate (KHCO <sub>3</sub> )	10 mM	Buffering capacity
Disodium EDTA (Na <sub>2</sub> EDTA)	0.1 mM	Chelates divalent cations; prevents clumping
Sterile distilled water	—	Solvent

### Note

This composition matches the standard formulation used in immunology workflows (commonly referred to as “ACK lysis buffer” or “RBC lysis buffer”).

## Preparation

### Reagent stocks

Reagent	Stock concentration	Notes
NH Cl	Powder	Prepare fresh solution
KHCO	Powder	CO -reactive; store desiccated
Na EDTA (disodium)	Powder	Use ultrapure cell-culture grade
Sterile distilled water	—	For dissolution

## Preparation of 1 L ACK lysis buffer

1. Add the following to a 1 L glass bottle or beaker:
  - **8.02 g NH Cl** (150 mM)
  - **1.00 g KHCO** (10 mM)
  - **0.037 g Na EDTA** (0.1 mM)
2. Add ~800 mL sterile distilled water.
3. Stir or gently swirl until fully dissolved.
4. Adjust the final volume to **1 L** with sterile distilled water.
5. Filter-sterilize using a **0.22 µm PES or PVDF filter**.
6. Aliquot if desired.

## pH adjustment

- Typically **no pH adjustment is required**; the solution should be at pH ~7.2–7.4.
- If necessary, adjust minimally with sterile HCl or NaOH.

## Storage and stability

- Store at **4 °C** for up to **3 months**.
- Aliquots stored sterilely remain stable for common immunology workflows.
- **Do not freeze**; precipitation and performance changes may occur.

## Reagent details

Reagent	Supplier	Cat. #	Notes
Ammonium chloride	Various	—	Analytical or cell-culture grade
Potassium bicarbonate	Various	—	Store tightly sealed
Disodium EDTA (Na EDTA)	Various	—	Chelates divalent cations
Sterile distilled water	—	—	Solvent

## Safety

- Wear gloves and eye protection; NH Cl is an irritant.
- Dispose of unused buffer and erythrocyte-containing waste as biological/chemical waste.
- Prepare and filter-sterilize the buffer in a biosafety cabinet when used for primary tissue processing.

## Version history

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