ACK Red Blood Cell Lysis Buffer

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Protocol ID: BUF-ACK-LYSIS-001
Version: v1.1
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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). Under brief incubation, the buffer efficiently removes red blood cells while preserving leukocyte viability and integrity.

Working buffer composition

Component	Final concentration	Notes
Ammonium chloride (NH Cl)	X mM	Primary erythrocyte-lysing component
Potassium bicarbonate (KHCO)	X mM	Buffering capacity
EDTA	X mM	Chelates divalent cations; reduces cell clumping
Sterile distilled water	—	Solvent
pH	7.2	Adjusted after dissolution

> **Note**
>
> This formulation reflects the standard ACK buffer used in immunology workflows, with explicit pH adjustment to ensure reproducibility across batches.

Preparation

Reagents

Reagent	Form	Notes
NH Cl	Powder	Analytical or cell-culture grade
KHCO	Powder	Hygroscopic; store tightly sealed
EDTA	Powder	Cell-culture grade
Distilled water	—	Sterile (bidest)
HCl	—	For pH adjustment

Preparation of 1 L ACK lysis buffer

1. Use a clean **1 L glass bottle** or beaker.
2. Add the following dry reagents:
    - **8.26 g NH Cl**
    - **1.00 g KHCO**
    - **0.037 g (37 mg) EDTA**
3. Add **1 L sterile distilled (bidest) water**.
4. Mix thoroughly by stirring or gentle swirling until all components are fully dissolved.
5. Measure pH and **adjust to pH 7.2** using dilute HCl.
6. **Sterile-filter** the solution using a **0.22 µm vacuum filter unit**.
7. Aliquot if desired under sterile conditions.

Labelling

Clearly label each bottle or aliquot with: - Buffer name: **ACK Lysis Buffer** - Date of preparation - Preparer initials - **Sterile** - **pH adjusted to 7.2**

Storage and stability

- Store at **4 °C**.
- Stable for up to **3 months** when sterile.
- **Do not freeze**, as freezing may alter buffer performance.

Safety and handling

- Wear gloves and eye protection; ammonium chloride is an irritant.
- Dispose of unused buffer and erythrocyte-containing waste according to biological/chemical waste regulations.
- For use in primary tissue processing, preparation and sterile filtration should be performed using appropriate aseptic technique.

Version history

Version	Date	Author	Changes
v1.1	2026-01-09	Dillon Corvino	Corrected concentrations, explicit pH adjustment, clarified sterility and labelling requirements
v1.0	2025-11-21	Dillon Corvino	Initial Quarto buffer document for ACK lysis buffer