# 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 |