ACK Red Blood Cell Lysis Buffer

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Protocol ID: BUF-ACK-LYSIS **Version:** v1.2 **Author:** Dillon Corvino **Date:** 2026-09-18

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.

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Linked protocols

- `MUS-IEL-ISO`
- `MUS-SPL-DIS`

- `MUS-LNG-DIG`
- `MUS-BM-ISO`
- `MUS-BLD-PRC`
- `MUS-TMR-DIG`
- `MUS-LIV-DIG`

Working buffer composition

Component	Final concentration	Notes
Ammonium chloride (NH_4Cl)	X mM	Primary erythrocyte-lysing component
Potassium bicarbonate ($KHCO_3$)	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

A. Preparation of stock (if applicable)

Not applicable — prepared directly as the working buffer.

B. Preparation of working buffer

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_4Cl**
    - **1.00 g $KHCO_3$**
    - **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 um 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.

Reagent details

Component	Supplier	Cat#	Notes
NH4Cl	[TBD]	[TBD]	Powder; analytical or cell-culture grade
KHCO3	[TBD]	[TBD]	Powder; hygroscopic — store tightly sealed
EDTA	[TBD]	[TBD]	Powder; cell-culture grade
Distilled water	[TBD]	[TBD]	Sterile (bidest)
HCl	[TBD]	[TBD]	For pH adjustment

Safety (brief)

- 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
v1.0	2025-11-21	Dillon Corvino	Initial Quarto buffer document for ACK lysis buffer
v1.1	2026-01-09	Dillon Corvino	Corrected concentrations, explicit pH adjustment, clarified sterility and labelling requirements
v1.2	2026-09-18	Dillon Corvino	Migrated to shared summary:/helper architecture (buffer/protocol redesign).