

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 ₄ Cl) | 150 mM | Primary erythrocyte-lysing component |
| Potassium bicarbonate (KHCO ₃) | 10 mM | Buffering capacity |
| Disodium EDTA (Na ₂ 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. |