ACK Red Blood Cell Lysis Buffer

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