

RBC Lysis Buffer (Hybrimax / Commercial)

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Protocol ID: BUF-RBC-HYB-v1.0

Version: v1.0

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Purpose

This buffer is used to lyse red blood cells in single-cell suspensions derived from liver (and other tissues, if applicable) while aiming to preserve leukocyte populations for downstream analysis.

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Linked protocols

This buffer is used in:

- **Mouse Liver Dissociation to Single-Cell Suspension – LIV-001 (v1.0)**

- Additional protocols where RBC lysis is required.

Composition

This protocol assumes the use of a **commercially supplied RBC lysis buffer**, such as:

- **HybriMax RBC lysis buffer** (Sigma), used at 1× working strength.

If an alternative supplier is used (e.g. BioLegend RBC Lysis Buffer), document the product and use according to manufacturer instructions, adapting incubation times as internally validated.

Preparation

A. From HybriMax (ready-to-use formulation)

1. Confirm whether the commercial HybriMax RBC lysis buffer is supplied as **ready-to-use (1×)** or **concentrate (e.g. 10×)**.
2. If supplied as **1× solution**:
 - Aliquot into smaller working volumes (e.g. 5–10 mL) to avoid repeated bottle opening.
 - No further dilution is required.
3. If supplied as **concentrate (e.g. 10×)**:
 - Dilute with sterile water or buffer as per manufacturer's instructions to make **1× RBC lysis solution**.
 - Mix thoroughly.
4. Label aliquots with:
 - “RBC Lysis Buffer (HybriMax)”
 - 1× or stock concentration
 - Date of preparation
 - Version (BUF-RBC-HYB-v1.0)

i Note

The liver protocol LIV-001 uses **0.5 mL of 1× RBC lysis buffer per sample** after Percoll enrichment, with a **7-minute** incubation at room temperature.

Storage and stability

- Store the commercial RBC lysis buffer according to manufacturer's instructions (commonly at **2–8 °C**).
- Keep tightly closed to avoid evaporation and contamination.
- Aliquoted 1× working solution should also be stored at **2–8 °C** and used within the period recommended by the manufacturer or internal validation.
- Do not freeze unless explicitly supported by the manufacturer.

Reagent details

| Component | Supplier | Cat# | Notes |
|---------------------------|----------|-------|---|
| HybriMax RBC lysis buffer | [TBD] | [TBD] | Commercial RBC lysis buffer; used at 1× working concentration |

(If using a different brand, add a row or update Supplier/Cat# accordingly.)

Safety (brief)

- RBC lysis buffers typically contain detergents and may contain **sodium azide** or other toxic components.
- Handle with gloves and standard lab PPE.
- Avoid contact with eyes and skin; consult the manufacturer's safety data sheet (SDS).
- Dispose of RBC-lysis waste and contaminated consumables in accordance with local chemical and biohazard regulations.

Version history

| Version | Date | Author | Change summary |
|---------|------------|----------------|---------------------------|
| v1.0 | 2025-11-20 | Dillon Corvino | Initial buffer definition |