RBC Lysis Solution 10X

REF	Σ	
RBC10X-50ML	50 ml	RUO

1. PRODUCT DESCRIPTION

RBC Lysis Solution 10X is a buffered, concentrated (10X) ammonium chloride-based lysing reagent designed for the selective lysis of red blood cells in human peripheral blood and bone marrow samples.

When diluted to a 1X working concentration and used according to the recommended protocol, this reagent effectively lyses erythrocytes following monoclonal antibody staining, facilitating the analysis of leukocyte populations by flow cytometry.

The formulation ensures optimal preservation of leukocyte morphology and surface antigen expression, resulting in clear light scatter separation between leukocytes and red blood cell debris. RBC Lysis Solution 10X does not contain any fixative agents, allowing leukocytes to remain viable after lysis, which is essential for downstream applications requiring live cells.

2. RECOMMENDED USAGE

Immunostep's RBC Lysis solution, is intended for the lysis of whole red blood cells. This reagent works properly with samples of 4-11 x 10 3 leukocytes per microliter, usually this is equivalent to 100 µl of normal human or mouse whole blood sample. For samples with high cell number, dilute it with PBS to obtain the correct concentration.

This product is used as red blood cell (RBC) lysis buffer and is supplied as a 10X solution. It should be diluted to 1X in distilled water before using. The pH of the 1X solution should fall within the range of pH 7.1-7.4. Adjust the pH if necessary. Warm the 1X solution to room temperature prior to use. Samples must be prepared as single cell suspension in an appropriate anticoagulant (EDTA is recommended) tube. For professional use only.

Presentation: liquid.

Storage instruction: Store RBC lysis buffer between 2°C and 8°C.

3. REAGENTS PROVIDED

50 ml of 10X concentrate will yield a quantity of 1X solution that is sufficient to lyse 250 samples.

4. RECOMMENDATION AND WARNINGS

This product contains sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded. These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop.

Do not use after expiration date stamped on vial.

Store the prepared 1X Red Blood Cell Lysis Solution at room temperature. Discard unused solution at the end of the day.

Before acquiring samples, adjust the discriminator (threshold) to minimize debris.

5. RED BLOOD CELLS LYSIS PROTOCOL

1. Prepare 1X working solution diluting Red Blood Cell Lysis Solution (10X) 1:10 with distilled water (dH 2 O).
   For example, dilute 10 mL of RBC Lysis Solution (10X) with 90 mL of dH 2 O.
2. For each sample, after the incubation process with the antibodies, add 2 ml of 1X working RBC lysis solution.
3. Mix gently with a vortex mixer
4. Incubate in the dark at room temperature (20-25°C) for 15 minutes or at 4 °C for 30 minutes.
5. Centrifuge at 540xg for 5 minutes and carefully aspirate the supernatant so as not to touch the cell pellet.
6. Resuspend the cell pellet in an appropriate buffer and proceed to further applications.

6. RED BLOOD CELLS LYSIS PROTOCOL FOR BULK LYSIS

Sample processing procedure

1. **Preparation of RBC Lysis Working Solution**
   Prepare a 1X working solution by diluting RBC Lysis Solution (10X) at a ratio of 1:10 with deionized water (dH_2O)
   Example: Mix 10 mL of RBC Lysis Solution (10X) with 90 mL of dH_2O

2. **Sample Transfer**
   Transfer an adequate volume of sample into a 50 mL conical-bottom Falcon tube to ensure a minimum of 10×10^6 nucleated cells per tube for staining. Use at least 2 mL of sample per 50 mL of RBC lysis solution. If the cell concentration is low, use multiple tubes accordingly.

3. **Addition of RBC Lysis Solution**
   Fill the tube to a final volume of 50 mL with the prepared 1X RBC Lysis Solution at room temperature.

4. **Mixing and Incubation**
   Mix thoroughly by inversion and incubate for 15 minutes on a roller or sample shaker at room temperature (20–25 °C), protected from light.

5. **Centrifugation**
   Centrifuge at 800 × g for 10 minutes. Carefully aspirate the supernatant using a Pasteur pipette or vacuum system, leaving approximately 300 µL of cell suspension without disturbing the pellet.

6. **First Wash**
   Add 2 mL of washing buffer and resuspend the pellet vigorously (preferably using a vortex mixer). Fill the tube to 50 mL with washing buffer and mix by inverting the tube 3–5 times.

7. **Second Centrifugation**
   Centrifuge at 800 × g for 5 minutes and aspirate the supernatant carefully.

8. **Transfer to FACS Tube**
   Resuspend the pellet in 2 mL of washing buffer and transfer to a 5 mL round-bottom polystyrene Falcon tube. Rinse the original 50 mL tube with 2 mL of washing buffer to recover residual cells and add to the FACS tube.

9. **Third Centrifugation**
   Centrifuge at 540 × g for 5 minutes. Aspirate the supernatant, leaving at least 300 µL of residual volume.

10. **Cell Concentration Adjustment**
    If multiple tubes were used, combine the cell suspensions. Adjust the final concentration to 1×10^5 cells/µL using washing buffer.

11. **Sample Allocation for Staining**
    Allocate 100 µL (10×10^6 cells) of the sample per staining tube.

12. Continue with the standard staining protocol for surface and/or intracellular markers as required.

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services.

(tech@immunostep.com)

Please, refer to http://immunostep.com/content/31-support for technical information.

7. WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

8. EXPLANATION OF SYMBOLS

REF	Catalog reference
Σ	Contains sufficient for <n> test
	Regulatory Status
RUO	Research Use Only
	Manufacturer

9. MANUFACTURED BY:

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