

Contents

1. Description
 - 1.1 Background information
 - 1.2 Applications
2. Protocol
 - 2.1 Preparation of 1× Red Blood Cell Lysis Solution
 - 2.2 Red blood cell lysis with washing step
 - 2.3 Red blood cell lysis without washing step
3. Example

1. Description

Components	50 mL Red Blood Cell Lysis Solution (10×)
Capacity	For 50 mL of whole blood.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

Red Blood Cell Lysis Solution (10×) has been developed for the lysis of red blood cells to ensure optimal lysis of erythrocytes with minimal effect on all cell types obtained from tissue samples or blood. A washing step after erythrocyte lysis is optional, depending on the application. The solution is suitable for lysis of erythrocytes in single-cell suspensions of human, mouse, or rat origin.

1.2 Applications

- Lysis of erythrocytes in human peripheral blood or bone marrow.
- Lysis of erythrocytes in mouse or rat whole blood.
- Lysis of erythrocytes in single-cell suspensions obtained from different tissues.

2. Protocol

2.1 Preparation of 1× Red Blood Cell Lysis Solution

1. Dilute Red Blood Cell Lysis Solution (10×) 1:10 with double-distilled water (ddH₂O), for example, dilute 1 mL of Red Blood Cell Lysis Solution (10×) with 9 mL of ddH₂O.

▲ **Note:** Do not dilute with deionized water.

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

2.2 Red blood cell lysis with washing step

1. Dilute one volume of cell suspension with 10 volumes of 1× Red Blood Cell Lysis Solution.

2. When working with blood samples:
Vortex for 5 seconds and incubate for 10 minutes at room temperature.

▲ **Note:** If the sample is not transparent red after 10 minutes, incubate for another 2 minutes.

When working with tissue samples:

Vortex for 5 seconds and incubate for 2 minutes at room temperature.

3. Centrifuge at 300×g for 5 minutes (tubes up to 5 mL) or 10 minutes (15 mL or 50 mL tubes) at room temperature. Aspirate supernatant completely.
4. Resuspend the cell pellet in an appropriate buffer and proceed to further applications.

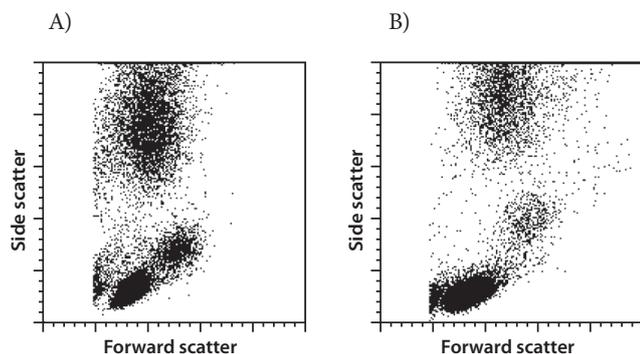
2.3 Red blood cell lysis without washing step

▲ This procedure is recommended for red blood cell lysis after fluorescent staining of the cell suspension prior to flow cytometric analysis, and if a volume reduction is not required.

1. Dilute one volume of cell suspension with 10 volumes of 1× Red Blood Cell Lysis Solution.
2. Vortex for 5 seconds and incubate for 10 minutes in the dark at room temperature.
3. Store sample on ice and analyse within one hour.

3. Example

Erythrocytes in human peripheral blood were lysed with Red Blood Cell Lysis Solution followed by a washing step (A), or without washing step (B) and analyzed by flow cytometry. Dead cells were excluded from the analysis based on propidium iodide fluorescence.



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