

## Red Blood Cell Lysis Buffer Plus

### Introduction

Red Blood Cell Lysis Buffer Plus is used for optimal lysis of erythrocytes in whole blood or tissues from human, mouse, rat and other mammals.

The buffer contains ammonium chloride, which can efficiently lyse erythrocytes with minimal effect on lymphocytes. However, the buffer is not suitable for lysis of nucleated erythrocytes, such as the erythrocytes of birds and poultry. The buffer is sterile, and the lysed samples can be used for further analysis such as nucleic acid or protein extraction, cell culture and flow cytometry. This reagent has been optimized to deliver better lysing effects and shorter lysis time compared to conventional Red Blood Cell Lysis Buffer (Cat. No: K1169).

### Components and Storage

| Components                       | 100 mL | 500 mL             | Storage |
|----------------------------------|--------|--------------------|---------|
| Red Blood Cell Lysis Buffer Plus | 100 mL | 500 mL             | 4°C     |
| Shipping: Blue ice               |        | Shelf life: 1 year |         |

### Protocol

#### 1. Lysis of tissue cells

- 1) Digest fresh tissues with trypsin or collagenase to prepare single-cell suspension. Centrifuge the cell suspension and discard the supernatant.
- 2) Add Red Blood Cell Lysis Buffer Plus at a volume 3-5 times that of the cell pellet, gently pipette to mix evenly, and lyse cells for 2 min. This step can be performed at room temperature or 4°C; lysis at 4°C yields better results.

**Note:** Incubation time may vary depending on the experimental application.

- 3) Centrifuge at 400-500 g for 5 min, aspirate the supernatant. Centrifugation at 4°C is recommended for optimal performance.

**Note:** If incomplete red blood cell lysis is observed, repeat steps 2 and 3 once. Trace residual red blood cells generally do not interfere with downstream experiments.

- 4) Resuspend 1mL pellet with at least 5 mL PBS, HBSS, saline or serum-free medium, centrifuge at 400-500 g for 2-3 min and aspirate the supernatant. It is better to perform this step at 4°C. (This washing step

may be repeated once for a total of 1-2 washes.)

5) Resuspend the pellet with an appropriate buffer for the downstream analysis.

## 2. Lysis of mouse or human blood

- 1) Take fresh anticoagulated whole blood, centrifuge at 400-500 g for 5 min and discard the supernatant.
- 2) Add Red Blood Cell Lysis Buffer Plus at a volume 6-10 times that of the cell pellet, gently pipette to mix evenly, and lyse cells for 2 min. This step can be performed at room temperature or 4°C; lysis at 4°C yields better results.

**Note:** Incubation time may vary depending on the experimental application. For mouse blood, it is enough to incubate 1-2 min. But for human blood, it is better to incubate 4-5 min with occasional shaking.

- 3) Centrifuge at 400-500 g for 5 min, aspirate the red supernatant. Centrifugation at 4°C is recommended for optimal performance.

**Note:** If incomplete red blood cell lysis is observed, repeat steps 2 and 3 once. Trace residual red blood cells generally do not interfere with downstream experiments.

- 4) Resuspend 1mL pellet with at least 5 mL PBS, HBSS, saline or serum-free medium, centrifuge at 400-500 g for 2-3 min and aspirate the supernatant. It is better to perform this step at 4°C. (This washing step may be repeated once for a total of 1-2 washes.)
- 5) Resuspend the pellet with an appropriate buffer for the downstream analysis.

**Note:** For trace blood samples, step 1 can be omitted. Directly add Red Blood Cell Lysis Buffer Plus at a volume 10 times the volume of whole blood, and lyse samples for 4-5 min at room temperature or 4°C. For mouse blood, it is enough to incubate 4-5 min. But for human blood, it is better to incubate 10 min with occasional shaking (no more than 15 min). Follow the remaining steps as described above.

## Note

1. The buffer is sterile, please pay attention to the aseptic operation. It is optimal to perform in a biosafety cabinet.
2. For total RNA extraction, the lysed samples using this buffer do not need DEPC treatment.
3. For your safety and health, please wear lab coats and gloves during the experiment.

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