Red Blood Cell Lysis Buffer

Introduction

Red Blood Cell Lysis Buffer 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.

Components and Storage

Component	K1169-100 mL	K1169-500 mL
Red Blood Cell Lysis Buffer	100 mL	500 mL
Store the product at 4°C. Stable for 1 year.		

Protocol

(一)Lysis of tissue cells

1. Soak fresh tissues in trypsin or collagenase to obtain a single cell suspension. Pellet the cells by centrifugation and aspirate the supernatant.
2. Add 3-5 mL the red blood cell lysis buffer per 1 mL of the pellet, gently resuspend the cells and incubate for 1-2 min. This step can be performed at room temperature or 4°C, but 4°C is better.
3. Centrifugation (400-500 g) for 5 min, aspirate the red supernatant. This step can be performed at room temperature or 4°C, but 4°C is better.

***Note:** Generally, a small number of red blood cell does not disturb the further analysis. If necessary, repeat steps 2 to 3 for a second round of lysis.

4. Resuspend 1mL pellet with at least 5 mL PBS, HBSS, saline or serum-free medium, centrifugation (400-500 g) for 2-3 min and aspirate the supernatant. It is better to perform this step at 4°C. (This step can be optional repeated, and totally wash 1-2 times)
5. Resuspend the pellet with an appropriate buffer for the further analysis.

(二)Lysis of tissue cells without washing

1. Soak fresh tissues in trypsin or collagenase to obtain a single cell suspension. Pellet the cells by centrifugation and aspirate the supernatant.
2. Add 5 mL the red blood cell lysis buffer per 1 mL of the pellet, gently resuspend the cells and incubate for 1-2 min. This step can be performed at room temperature or 4°C, but 4°C is better.
3. Resuspend pellet with 15-20 mL PBS, HBSS, saline or serum-free medium, gently mix the cells, centrifugation (400-500 g) for 2-3 min and aspirate the red supernatant. It is better to perform this step at 4°C.

***Note:** Generally, a small number of red blood cell does not disturb the further analysis. If necessary, repeat steps 2 to 3 for a second round of lysis.

4. Resuspend the pellet with an appropriate buffer for the further analysis.

***Note:** This experiment is easier without washing, but the washing effect is slightly inferior and large centrifugal tubes are required.

(三)Lysis of mouse or human blood

1. Collect fresh whole blood into a tube containing anticoagulant, centrifugation (400-500 g) for 5 min and aspirate the supernatant.
2. Add 6-10 mL the red blood cell lysis buffer per 1 mL of the pellet, gently resuspend the cells and incubate for 1-2 min. This step can be performed at room temperature or 4°C, but 4°C is better.

***Note:** 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. Centrifugation (400-500 g) for 5 min, aspirate the red supernatant. This step can be performed at room temperature or 4°C, but 4°C is better.

***Note:** Generally, a small number of red blood cell does not disturb the further analysis. If necessary, repeat steps 2 to 3 for a second round of lysis.

4. Resuspend 1mL pellet with at least 5 mL PBS, HBSS, saline or serum-free medium, centrifugation (400-500 g) for 2-3 min and aspirate the supernatant. It is better to perform this step at 4°C. (This step can be optional repeated, and totally wash 1-2 times)
5. Resuspend the pellet with an appropriate buffer for the further analysis.

***Note:** For small amounts of blood samples, step 1 can be omitted and directly start from Step 2, but 10 mL the red blood cell lysate is needed for 1 mL blood samples and incubate at room temperature or 4°C for 4-5 min. For mouse blood, it is enough to incubate 1-2 min. But for human blood, it is better to incubate 10-15 min with occasional shaking (no more than 15 min).

(四)Lysis of mouse or human blood without washing

1. Add 10 mL the red blood cell lysis buffer per 1 mL of the blood, gently mix the cells and incubate for 4-5 min. This step can be performed at room temperature or 4°C, but 4°C is better.

***Note:** For mouse blood, it is enough to incubate 4-5 min. But for human blood, it is better to incubate 10-15 min with occasional

shaking (no more than 15 min).

2. Resuspend pellet with 20 mL PBS, HBSS, saline or serum-free medium, gently mix the cells.
3. Centrifugation (400-500 g) for 5 min and aspirate the red supernatant. It is better to perform this step at 4°C.

***Note:** Generally, a small number of red blood cell does not disturb the further analysis. If necessary, repeat steps 2 to 3 for a second round of lysis.

4. Resuspend the pellet with an appropriate buffer for the further analysis.

***Note:** This experiment is easier without washing, but the washing effect is slightly inferior and large centrifugal tubes are required.

Note

1. The buffer is sterile, please pay attention to the aseptic operation. It is optimal to perform in a clear bench.
2. For total RNA extraction, the lysed samples using this buffer do not need DEPC treatment.
3. To improve the significance of experimental results, please try to use fresh blood. If you find blood clots in samples, try to remove it. Use vacuum blood collection tubes containing anticoagulants to collect blood samples as much as possible.
4. For your safety and health, please wear lab coats and gloves during the experiment.

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