Tris-Ammonium Chloride Red Blood Cell Lysis Buffer

Cat #: A-CSH517

Size: 100mL,500mL

Storage: 2-8°C (12 months)

Product Description

There are multiple methods for removing red blood cells, and one of them is Tris-NH4Cl Red Blood Cell Lysis Buffer, also known as Tris-NH4Cl Lysis Buffer. It is a solution used to lyse and remove nucleated red blood cells from tissue samples or blood from humans, mice, or other mammals. The main active ingredient in Tris-NH4Cl Lysis Buffer is ammonium chloride. This buffer has been optimized to effectively lyse non-nucleated red blood cells while causing minimal damage to lymphocytes or other nucleated cells.

However, Tris-NH4Cl Lysis Buffer is not suitable for lysing and removing nucleated red blood cells from species like birds or poultry. It is not recommended to use this buffer for lysing similar types of cells. The lysis buffer is sterilized through filtration. Blood or tissue cell samples treated with Tris-NH4Cl Lysis Buffer can be used for subsequent cell culture, cell fusion, as well as extraction, analysis, and detection of nucleic acids or proteins using various routine methods..

Usage

(1) General procedures for tissue cell samples:

1. Preparation of cell suspension: Fresh tissue is subjected to digestion with enzymes such as trypsin or collagenase. The cell suspension is prepared using an appropriate method, and the supernatant is discarded after centrifugation.

2. Lysis: Add Tris-NH4Cl Lysis Buffer at a volume 3-5 times that of the cell pellet. Gently pipette and mix for 2-3 minutes for lysis. This step is preferably performed at 4° C but can also be done at room temperature.

3. Centrifugation: Centrifuge the cells at 300-400g for 10 minutes at 4° C in 100% fetal bovine serum. Discard the red supernatant. If a low-temperature centrifuge is not available, this step can also be performed at room temperature.

4. If incomplete red blood cell lysis is observed, repeat steps 2 and 3 once.

5. Wash: Add an appropriate amount of PBS, HBSS, physiological saline, or serum-free culture medium according to

specific experimental requirements. Gently mix and resuspend the pellet. Centrifuge at 300-400g for 3-5 minutes at 4° C, discard the supernatant. The centrifugation step can also be performed at room temperature. The volume of PBS, HBSS, physiological saline, or serum-free culture medium added should generally be more than 5 times the volume of the cell pellet.

6. If necessary, repeat step 5 once for a total of 1-2 washes.

7. Resuspend the cell pellet in an appropriate solution according to the experimental needs for counting, culture, or subsequent experiments.

(2) Rapid procedures for tissue cell samples (no washing required):

1. Preparation of cell suspension: Fresh tissue is subjected to digestion with enzymes such as trypsin or collagenase. The cell suspension is prepared using an appropriate method, and the supernatant is discarded after centrifugation.

2. Lysis: Add Tris-NH4Cl Lysis Buffer at a volume 5 times that of the cell pellet. Gently pipette and mix for 2-3 minutes for lysis. This step is preferably performed at 4°C but can also be done at room temperature.

3. Add 15-20 ml of PBS, HBSS, physiological saline, or serum-free culture medium. Gently mix.

4. Centrifugation: Centrifuge the cells at 300-400g for 5 minutes at 4°C in 100% fetal bovine serum. Discard the red supernatant. This step can also be performed at room temperature.

5. If incomplete red blood cell lysis is observed, repeat steps 2 to 4 once.

6. Resuspend the cell pellet in an appropriate solution according to the experimental needs for counting, culture, or subsequent experiments.

(3) General procedures for blood samples:

1. Obtain fresh anticoagulated blood and centrifuge at 400-500g for 5 minutes. Discard the supernatant.

2. Lysis: Add Tris-NH4Cl Lysis Buffer at a volume 6-10 times that of the cell pellet. Gently pipette and mix for 2-5 minutes for lysis. This step is preferably performed at 4°C but can also be done at room temperature. (Note: For mouse blood, 2-3 minutes of lysis is sufficient. For human peripheral blood, it is recommended to extend the lysis time to 4-5 minutes and gently shake during lysis to promote red blood cell lysis.)

3. Centrifugation: Centrifuge the cells at 300-400g for 10 minutes at 4°C in 100% fetal bovine serum. Discard the red supernatant. If a low-temperature centrifuge is not available, this step can also be performed at room temperature.

4. If incomplete red blood cell lysis is observed, repeat steps 2 and 3 once.

5. Wash: Add an appropriate amount of PBS, HBSS, physiological saline, or serum-free culture medium according to

specific experimental requirements. Gently mix and resuspend the pellet. Centrifuge at 300-400g for 3-5 minutes at 4°C, discard the supernatant. The centrifugation step can also be performed at room temperature. The volume of PBS, HBSS, physiological saline, or serum-free culture medium added should generally be more than 5 times the volume of the cell pellet.

6. Resuspend the cell pellet in an appropriate solution according to the experimental needs for counting, culture, or subsequent experiments. Note: For small or minimal blood samples, the first step can be omitted. Instead, add Tris-NH4Cl Lysis Buffer at a volume 10 times that of the blood sample for the second step and perform lysis at 4°C or room temperature for 4-15 minutes. For mouse blood, 4-5 minutes of lysis is sufficient. For human peripheral blood, it is recommended to extend the lysis time to 10 minutes, but usually not exceeding 15 minutes. During lysis, gently shaking the sample can promote red blood cell lysis.

(4) Rapid procedure for blood samples (no washing required):

1. Add Tris-NH4Cl Lysis Buffer at a volume 10 times that of fresh anticoagulated blood. Gently pipette and mix, and perform lysis for 4-15 minutes. This step is preferably performed at 4°C but can also be done at room temperature. (Note: For mouse blood, 4-5 minutes of lysis is sufficient. For human peripheral blood, it is recommended to extend the lysis time to 10 minutes, but usually not exceeding 15 minutes. During lysis, gently shaking the sample can promote red blood cell lysis.)

2. Add 20-30ml of PBS, HBSS, physiological saline, or serum-free culture medium. Gently mix.

3. Centrifuge the cells at 300-400g for 10 minutes in 100% fetal bovine serum. Discard the red supernatant. Centrifugation at 4°C yields better results.

4. If incomplete red blood cell lysis is observed, repeat steps 2 and 3 once.

5. Resuspend the cell pellet in an appropriate solution according to the experimental needs for counting, culture, or subsequent experiments.

Notes

1. The preparation of cell suspension should be based on specific experimental requirements and does not necessarily need to be prepared as a single-cell suspension.

2. If the subsequent experiments involve cell culture, sterile techniques should be observed, and it is preferable to perform the procedures within a laminar flow hood.

3. Whenever possible, centrifugation steps should be conducted at 4°C using a refrigerated centrifuge.

4. The main difference between the regular procedure and the rapid procedure lies in the washing step. The regular procedure includes an additional centrifugation step for washing, which can reduce the volume of washing solution used and improve the washing efficiency without requiring large-volume centrifuge tubes. The rapid procedure omits one centrifugation step, resulting in slightly inferior washing efficiency and the need for larger-volume centrifuge tubes.

5. After centrifugation and washing, the presence of trace amounts of red blood cells typically does not affect subsequent analysis.

6. If the samples treated with Tris-NH4Cl Lysis Buffer are intended for total RNA extraction, there is no need to use DEPC-treated solutions during cell handling. RNase removal is not necessary for this step.

7. For your safety and health, please wear laboratory attire and disposable gloves during the procedures.

Disclaimer

For your safety and health, please wear a lab coat and disposable gloves while handling.