Tris-Ammonium Chloride Red Blood Cell Lysis Buffer (Sterile)

Cat. No.: T1509568 | Pack size: 100 mL; 500 mL | Storage: Store at 2-8°C

Product Description

There are various methods for removing red blood cells. Tris-Ammonium Chloride Red Blood Cell Lysis Buffer (Tris-NH_4Cl Red Blood Cell Lysis Buffer), also referred to as Tris-NH_4Cl Lysis Buffer, is a solution designed to lyse and eliminate enucleated red blood cells from tissue samples or blood of humans, mice, or other mammals. Its main active component is ammonium chloride. With an optimized formulation, Tris-NH_4Cl Lysis Buffer can lyse enucleated red blood cells while causing almost no damage to lymphocytes or other nucleated cells.

This lysis buffer has poor efficacy in lysing and removing nucleated red blood cells, such as those from birds or avian species, and is not recommended for use with such cells. The lysis buffer is sterilized by filtration. Blood or tissue cell samples treated with Tris-NH_4Cl Lysis Buffer can be used for subsequent experiments including cell culture, cell fusion, nucleic acid or protein extraction, as well as various routine analyses and detections.

Operating Procedures

(1) Routine Operation for Tissue Cell Samples

1. Prepare cell suspension: Digest fresh tissues with trypsin, collagenase, or other suitable enzymes, prepare a cell suspension using an appropriate method, and centrifuge to discard the supernatant.

2. Lysis: Add 3-5 volumes of Tris-NH_4Cl Lysis Buffer relative to the cell pellet volume, gently pipette to mix thoroughly, and incubate for lysis at 2-3 minutes. This step is optimally performed at 4°C, but can also be carried out at room temperature.

3. Centrifugation: Resuspend the cells in 100% fetal bovine serum, centrifuge at 300-400×g for 10 minutes at 4°C, and discard the red supernatant. If a low-temperature centrifuge is unavailable, this step can be performed at room temperature.

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

5. Washing: Add an appropriate volume of PBS, HBSS, normal saline, or serum-free medium according to specific experimental requirements, gently mix to resuspend the pellet. Centrifuge at 300-400×g for 3-5 minutes at 4°C, and discard the supernatant. This centrifugation step can also be performed at room temperature. The volume of added PBS, HBSS, normal saline, or serum-free medium should generally be more than 5 times the volume of the cell pellet.

6. If necessary, repeat step 5 once, with a total of 1-2 washing cycles.

7. Resuspend the cell pellet with an appropriate solution according to experimental needs, and proceed with subsequent experiments such as cell counting and culture.

(2) Rapid Operation for Tissue Cell Samples (No Washing Required)

1. Prepare cell suspension: Digest fresh tissues with trypsin, collagenase, or other suitable enzymes, prepare a cell suspension using an appropriate method, and centrifuge to discard the supernatant.

2. Lysis: Add 5 volumes of Tris-NH_4Cl Lysis Buffer relative to the cell pellet volume, gently pipette to mix thoroughly, and incubate for lysis at 2-3 minutes. This step is optimally performed at 4°C, but can also be carried out at room temperature.

3. Add 15-20 mL of PBS, HBSS, normal saline, or serum-free medium, and mix gently.

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

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

6. Resuspend the cell pellet with an appropriate solution according to experimental needs, and proceed with subsequent experiments such as cell counting and culture.

(3) Routine Operation for Blood Samples

1. Take fresh anticoagulated blood, centrifuge at 400-500×g for 5 minutes, and discard the supernatant.

2. Lysis: Add 6-10 volumes of Tris-NH_4Cl Lysis Buffer relative to the cell pellet volume, gently pipette to mix thoroughly, and incubate for lysis at 2-5 minutes. This step is optimally performed at 4°C, but can also be carried out at room temperature. (Important Note: For mouse blood, 2-3 minutes of lysis is sufficient; for human peripheral blood, it is advisable to extend the lysis time to 4-5 minutes with gentle shaking during lysis to facilitate red blood cell lysis.)

3. Centrifugation: Resuspend the cells in 100% fetal bovine serum, centrifuge at 300-400×g for 10 minutes at 4°C, and discard the red supernatant. If a low-temperature centrifuge is unavailable, this step can be performed at room temperature.

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

5. Washing: Add an appropriate volume of PBS, HBSS, normal saline, or serum-free medium according to specific experimental requirements, gently mix to resuspend the pellet. Centrifuge at 300-400×g for 3-5 minutes at 4°C, and discard the supernatant. This centrifugation step can also be performed at room temperature. The volume of added PBS, HBSS, normal saline, or serum-free medium should generally be more than 5 times the volume of the cell pellet.

6. Resuspend the cell pellet with an appropriate solution according to experimental needs, and proceed with subsequent experiments such as cell counting and culture. Note: For trace or small-

volume blood samples, step 1 can be omitted. Add 10 volumes of Tris-NH_4Cl Lysis Buffer relative to the blood volume and perform step 2, incubating for 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 advisable to extend the lysis time to 10 minutes (usually not exceeding 15 minutes) with appropriate shaking during lysis to facilitate red blood cell lysis.

(4) Rapid Operation for Blood Samples (No Washing Required)

1. Add 10 volumes of Tris-NH_4Cl Lysis Buffer to fresh anticoagulated blood, gently pipette to mix thoroughly, and incubate for lysis at 4°C or room temperature for 4-15 minutes. (Important Note: For mouse blood, 4-5 minutes of lysis is sufficient; for human peripheral blood, it is advisable to extend the lysis time to 10 minutes (usually not exceeding 15 minutes) with appropriate shaking during lysis to facilitate red blood cell lysis.)

2. Add 20-30 mL of PBS, HBSS, normal saline, or serum-free medium, and mix gently.

3. Resuspend the cells in 100% fetal bovine serum, centrifuge at 300-400×g for 10 minutes (centrifugation at 4°C yields better results), and discard the red supernatant.

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

5. Resuspend the cell pellet with an appropriate solution according to experimental needs, and proceed with subsequent experiments such as cell counting and culture.

Precautions

1. When preparing the cell suspension, it is not necessary to prepare a single-cell suspension unless required by specific experiments.

2. If subsequent experiments involve cell culture, aseptic techniques should be strictly followed, and operations should be performed in a clean bench as much as possible.

3. Centrifugation steps are preferably carried out using a low-temperature centrifuge set at 4°C.

4. The difference between routine and rapid procedures lies in that the routine procedure includes an additional centrifugation step for washing, which saves washing buffer consumption, achieves better washing efficiency, and does not require large-volume centrifuge tubes. In contrast, the rapid procedure omits one centrifugation step, resulting in slightly reduced washing efficiency and requiring large-volume centrifuge tubes.

5. After centrifugation and washing, trace amounts of residual red blood cells generally do not interfere with subsequent detections.

6. If samples treated with Tris-NH_4Cl Lysis Buffer are to be used for total RNA extraction, there is no need to use DEPC-treated solutions during the operation, i.e., RNase removal is not required in this process.

7. For your safety and health, wear a lab coat and disposable gloves during operation.

8. This product is for research use only and is prohibited for any other purposes.

Specifications

Attribute	Value
Synonyms	Tris-Ammonium Chloride Red Blood Cell Lysis Buffer | Tris-NH_4Cl Red Blood Cell Lysis Buffer | Tris-NH_4Cl Lysis Buffer
Specifications & Purity	sterile-filtered, BioReagent, sterile
Stability And Storage	Store at 2-8°C long term (12 months).
Storage Conditions	Store at 2-8°C
Shipped In	Wet ice This product requires cold chain shipping. Ground and other economy services are not available.

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