ACK Red Blood Cell Lysis Buffer (Sterile)

Cat #: A-CSH516

Size: 100mL,500mL

Storage: 2-8°C (12 months)

Product Description

ACK Lysis Buffer is a solution used to lyse and remove nucleated red blood cells from blood or tissue samples of humans or mice. This product has been optimized to effectively lyse red blood cells while minimizing damage to lymphocytes and other nucleated cells. It is not suitable for lysing red blood cells with nuclei, such as those from birds or poultry. The product has undergone sterile processing, and the processed blood or tissue cell samples can be used for subsequent primary cultures, cell fusion, as well as extraction of nucleic acids or proteins, and various routine analyses and tests.

Usage

For tissue cell samples:

1. Fresh tissue is digested using collagenase or pancreatin, or other appropriate methods to disperse cells into a suspension, and the supernatant is discarded by centrifugation.

2. Add 3-5 times the volume of the cell pellet of ACK Lysis Buffer, gently pipette and mix, and lyse for 1-2 minutes. For example, if the volume of the cell pellet is 1 ml, add 3-5 ml of ACK Lysis Buffer. This step can be performed at room temperature or 4° C.

3. Centrifuge at 400-500g for 5 minutes, discard the red supernatant. Centrifugation at 4° C yields better results.

4. If incomplete lysis of red blood cells is observed, repeat steps 2 and 3. Usually, a minimal amount of red blood cells does not affect subsequent assays.

5. Wash 1-2 times: Add an appropriate amount of PBS, HBSS, physiological saline, or serum-free culture medium, resuspend the pellet, centrifuge at 400-500g for 2-3 minutes, discard the supernatant. Repeat the wash once, for a total of 1-2 washes. The volume of the wash solution should typically be at least 5 times the volume of the cell pellet. Centrifugation at 4° C yields better results.

6. Resuspend the cell pellet in an appropriate solution according to experimental needs and proceed with cell counting or other subsequent experiments.

Fast operation steps for tissue cell samples without the need for washing:

1. Fresh tissue is digested using collagenase or pancreatin, or other appropriate methods to disperse cells into a suspension, and the supernatant is discarded by centrifugation.

2. For a 0.2 ml cell pellet, add 1 ml of ACK Lysis Buffer, gently pipette and mix, and lyse for 1-2 minutes. This step can be performed at room temperature or 4°C.

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

4. Centrifuge at 400-500g for 5 minutes, discard the red supernatant. Centrifugation at 4°C yields better results.

5. If incomplete lysis of red blood cells is observed, repeat steps 2 and 3 once. Usually, a minimal amount of red blood cells does not affect subsequent assays.

6. Resuspend the cell pellet in an appropriate solution according to experimental needs and proceed with cell counting or other subsequent experiments.

Note: In the regular procedure, an additional centrifugation step is included during the washing process, which helps save the amount of wash solution used and improves the washing effect. It also eliminates the need for large-volume centrifuge tubes. The fast procedure eliminates one centrifugation step but may result in slightly poorer washing effect and requires large-volume centrifuge tubes.

For blood samples:

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

2. Add 6-10 times the volume of red blood cell lysis buffer to the cell pellet, gently pipette and mix, and lyse for 1-2 minutes. For example, if the volume of the cell pellet is 1 ml, add 6-10 ml of red blood cell lysis buffer. This step can be performed at room temperature or 4°C. Note: For mouse blood, 1-2 minutes of lysis is sufficient. For human peripheral blood, it is recommended to extend the lysis time to 4-5 minutes, and during the lysis process, occasional gentle shaking is recommended to promote red blood cell lysis.

3. Centrifuge at 400-500g for 5 minutes, discard the red supernatant. Centrifugation at 4°C yields better results.

4. If incomplete lysis of red blood cells is observed, repeat steps 2 and 3 once. Usually, a minimal amount of red blood

cells does not affect subsequent assays.

5. Wash 1-2 times: Add an appropriate amount of PBS, HBSS, physiological saline, or serum-free culture medium, resuspend the pellet, centrifuge at 400-500g for 2-3 minutes, and discard the supernatant. Repeat once if desired, for a total of 1-2 washes. The volume of wash solution used should be at least 5 times the volume of the cell pellet. Centrifugation at 4°C yields better results.

6. Resuspend the cell pellet in an appropriate solution according to experimental needs and proceed with cell counting or other subsequent experiments.

Note: For small or limited blood samples, the centrifugation and supernatant discarding steps can be skipped in the first step. Instead, directly add 10 times the volume of red blood cell lysis buffer in the second step and lyse for 4-5 minutes at room temperature or 4°C. 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 generally not exceeding 15 minutes. During the lysis process, occasional gentle shaking is recommended to promote red blood cell lysis. The subsequent steps remain the same.

Fast operation steps for blood samples without the need for washing:

1. Add 10 ml of red blood cell lysis buffer to every 1 ml of fresh anticoagulated blood, gently pipette and mix, and lyse for 4-5 minutes. This step can be performed at room temperature or 4°C. 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 generally not exceeding 15 minutes. During the lysis process, occasional gentle shaking is recommended to promote red blood cell lysis.

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

3. Centrifuge at 400-500g for 5 minutes, discard the red supernatant. Centrifugation at 4°C yields better results.

4. If incomplete lysis of red blood cells is observed, repeat steps 2 and 3 once. Usually, a minimal amount of red blood cells does not affect subsequent assays.

5. Resuspend the cell pellet in an appropriate solution according to experimental needs and proceed with cell counting or other subsequent experiments.

Note: In the regular procedure, an additional centrifugation step is included during the washing process, which helps save the amount of wash solution used and improves the washing effect. It also eliminates the need for large-volume centrifuge tubes. The fast procedure eliminates one centrifugation step but may result in slightly poorer washing effect

and requires large-volume centrifuge tubes.

Notes

1. This lysis buffer is a sterile product. Please ensure aseptic conditions and use this product within a laminar flow hood.

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

Disclaimer

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