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No. NBP2-29442 Red Blood Cell (RBC) Lysis Buffer

Contents: 10X RBC Lysis Buffer – 50 ml
Storage Condition: Stable for six months at room temperature or 4°C.

Description

The Red Blood Cell (RBC) Lysis Buffer is for optimal lysis of erythrocytes in single-cell suspensions of mouse hematopoietic tissues such as spleen and human peripheral blood. This buffer contains ammonium chloride, which lyses red cells with minimal effect on lymphocytes. Nucleated red blood cells are not effectively lysed with ammonium chloride. RBC lysis is not necessary when working with mouse thymus and lymph node.

Protocol

Dilution: Dilute 10X RBC Lysis Buffer to 1X in deionized water before use (1 ml of 10X RBC lysis buffer with 9 ml of deionized water). Bring to room temperature prior to use.

Lysis of mouse RBCs

1. Add 10 ml of 1X RBC Lysis Buffer per 1 ml of mouse blood.
2. Incubate at room temperature for 4-5 minutes.
3. Stop the reaction by diluting the solution with 20 ml of 1X PBS.
4. Spin the cells (300 x g) at 4°C for 10 minutes, aspirate supernatant.
5. Resuspend the pellet in 10 ml of 1X PBS.
6. Spin down cells (300 x g) for 10 minutes, aspirate supernatant.
7. Resuspend pellet in RPMI with 10% FBS.
8. Count cells.

Lysis of RBC from mouse splenocytes

1. Harvest mouse spleen and prepare a single cell suspension.
2. Pellet the cells by centrifugation (300 x g) at 4°C and aspirate the supernatant.
3. Resuspend the pellet in 10 ml of 1X Lysis Buffer.
4. Incubate at room temperature for 4-5 minutes.
5. Stop the reaction by diluting the solution with 20 ml of 1X PBS.
6. Spin the cells (300 x g) at 4°C for 10 minutes, aspirate supernatant.
7. Resuspend the pellet in 10 ml of 1X PBS.
8. Spin down cells (300 x g) for 10 minutes, aspirate supernatant.
9. Resuspend pellet in RPMI with 10% FBS.
10. Count cells.

Lysis of human RBC

1. Add 10 ml of lysis buffer per 1 ml of human blood.
2. Incubate for 15 minutes at room temperature (no more than 15 minutes).
3. Stop the reaction by diluting the solution with 20 ml of 1X PBS.
4. Spin the cells (300 x g) at 4°C, aspirate supernatant.
5. Resuspend the pellet in 10 ml of 1X PBS.
6. Spin down cells (300 x g) for 10 minutes, aspirate supernatant.
7. Resuspend pellet in appropriate media.
8. Count cells.

Note: *In general a small number of residual red blood cells does not interfere with the proliferation assays and can be gated out from flow cytometric analysis. However, if required, a second round of lysis can be performed.*