July 2014

Bulk Erythrocyte Lysing with Ammonium Chloride for Flow Cytometry Immunophenotyping

Scope

Use this method to detect cells bearing specific surface antigens. Begin by adding whole blood or bone marrow to ammonium chloride lysing solution to lyse erythrocytes under gentle hypotonic conditions while preserving the leukocytes. Then, wash the sample to remove cellular debris. Adjust the cellular concentration, as determined for antibody titrations. Stain cells with fluorochrome-conjugated monoclonal antibodies that bind specifically to cell surface antigens. Finally, analyze the cells by flow cytometry.

Reagents Required

Full Name	Short Name	Cat. No.
BD fluorochrome-conjugated antibodies to human cell surface antigens	Antibodies	
BD Pharm Lyse™ lysing buffer (10X concentrate)	BD Pharm Lyse buffer	555899
BD Pharmingen™ stain buffer (BSA) for use as wash buffer	Stain buffer	554657

Suggested Equipment

BD Vacutainer® blood collection tubes or equivalent

Falcon® disposable 12 x 75-mm capped polystyrene test tubes (5-mL tubes) or equivalent

Falcon® 15-mL conical centrifuge tubes or equivalent

Micropipettors with tips

Vortex mixer

Centrifuge

BD FACS™ brand flow cytometer

Procedural Notes

- Use EDTA as the anticoagulant. We have limited information concerning use of other anticoagulants such as heparin and ACD.
- If you use an ammonium chloride lysing solution other than BD Pharm Lyse buffer, add EDTA to a final concentration of 2 mM to minimize cell aggregation.
- When using monoclonal antibodies that react with serum immunoglobulins, wash blood samples with 1X PBS or physiological saline prior to staining and lysing.
- As an alternate wash buffer, you can use phosphate-buffered saline (PBS) with 0.1% sodium azide (Dulbecco's PBS without calcium, magnesium, or phenol red, pH 7.2 ±0.2). Filter the PBS through a 0.2-µm filter prior to use and store at 2°C–8°C.
- Samples with nucleated red blood cells can show incomplete lysis of red blood cells because ammonium chloride does not effectively lyse nucleated erythrocytes. This also can occur when assaying blood samples from patients with certain hematologic disorders in which red cells are difficult to lyse, such as myelofibrosis, sickle-cell anemia, thalassemia, and spherocytosis.
- You can resuspend cells before acquisition in a 1% paraformaldehyde solution prepared in PBS with 0.1% sodium azide. Store the solution at 2°C–8°C in amber glass for up to 1 week. However, we do not recommend this solution for use with tandem fluorochromes.
- Keep lysed, stained (unfixed) samples in the dark and at 2°C–8°C until acquisition. We recommend acquiring samples within 2 hours of staining.

WARNING: Formaldehyde is harmful by inhalation, in contact with skin, and if swallowed. It is irritating to eyes and skin. Exposure can cause cancer. Possible risks of irreversible effects. Can cause sensitization by skin contact. Keep locked up and out of the reach of children. Keep away from food, drink, and animal feeding stuff. Wear suitable protective clothing and gloves. If swallowed, seek medical advice immediately and show the container or label. Dispose of according to federal, state, and local regulations.

Procedure

Preparing Reagents

Each day that the procedure is done, prepare a fresh working reagent of BD Pharm Lyse buffer by diluting 1:10 with deionized or distilled water.

Collecting and Preparing Specimens

- Collect blood aseptically by venipuncture into a sterile BD Vacutainer blood collection tube (or equivalent). Follow the collection tube manufacturer's guidelines for the minimum volume of blood to be collected.
- Collect bone marrow aseptically into anticoagulant by accepted protocols.
- Store anticoagulated specimen at room temperature (20°C–25°C) until ready for lysing and staining. Refer to the appropriate package insert for storage restrictions prior to staining.

WARNING: All biological specimens and materials coming into contact with them are considered biohazards. Handle as if capable of transmitting infection and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

Lysing

1. Transfer 13 mL of room temperature 1X BD Pharm Lyse buffer to a labeled 15-mL conical tube.
2. Transfer 1 mL of well mixed blood or bone marrow to the tube containing the 13 mL of 1X BD Pharm Lyse buffer.
3. Cap, mix gently by inversion, and place on a tube rocker for 5 minutes.
4. Centrifuge at 300*g* for 5 minutes; verify that there is a cell pellet at the bottom of the tube.
5. Aspirate the supernatant without disturbing the pellet.
6. Dislodge the pellet with a Pasteur pipet or vortex gently.
7. Add 10 mL of 1X BD Pharm Lyse buffer to the tube, and cap, mix, and place on a tube rocker for 5 minutes.
8. Centrifuge at 300*g* for 5 minutes; verify that there is a pellet at the bottom of the tube.
9. Aspirate the supernatant without disturbing the pellet.
10. Dislodge the pellet with a Pasteur pipet or vortex gently.
11. Add 10 mL of stain buffer and cap the tube.
12. Centrifuge at 300*g* for 5 minutes; verify that there is a pellet at the bottom of the tube.
13. Aspirate the supernatant without disturbing the pellet.
14. Dislodge the pellet with a Pasteur pipet or vortex gently.
15. Add 1 mL of stain buffer (resuspending to the original 1-mL blood volume), cap the tube, and vortex gently.
16. Count the cells.
17. Adjust the cell count to ~10 x 10^4 per µL (~10 x 10^7 per mL) using stain buffer.

Staining

1. Transfer 100 µL of the cell suspension (~one million cells) to each 12 x 75-mm tube.
2. Incubate 100 µL of cell suspension with appropriately titered antibodies at room temperature (18°C–25°C), in the dark, for 15 minutes; mix at 7 minutes.
3. Wash once with 2 mL of stain buffer, centrifuging at 300*g* for 5 minutes.
4. Verify that there is a pellet at the bottom of the tube and aspirate the supernatant.
5. Resuspend in 300–400 µL of stain buffer for flow acquisition.
6. Mix samples thoroughly and acquire and analyze on a BD FACS brand flow cytometer.

References

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