



SUPPORT PROTOCOLS

Direct Immunofluorescence Staining of Whole Blood Using a Lyse/No-Wash Procedure

SCOPE

Use this method to detect cells bearing specific membrane antigens. Begin by adding whole blood to fluorochrome-conjugated monoclonal antibodies that bind specifically to cell surface antigens. Next, treat the stained sample with FACS Lysing Solution to lyse erythrocytes under gentle hypotonic conditions while preserving the leucocytes. Finally, analyze the cells by flow cytometry.

This protocol is optimized for use with MultiTEST and TriTEST reagents and with TruCOUNT Tubes to perform absolute counts.

Reagents and Equipment Required

Refer to the appropriate product labeling for intended use and precautions.

1. K₃ EDTA VACUTAINER blood collection tubes (BD Cat. No. 6457) or equivalent
2. Falcon disposable 12 x 75-mm capped polystyrene test tubes (BD Cat. No. 2058) or equivalent
3. Micropipettor with tips (BD Electronic Pipette, BD Cat. No. 343246 or equivalent)
4. BD fluorochrome-conjugated monoclonal antibodies to human cell surface antigens (for example, MultiTEST or TriTEST reagents). Refer to the appropriate reagent package insert for more information.
5. Vortex mixer
6. FACS Lysing Solution (10X) (BD Cat. No. 349202). For dilution instructions and warnings, refer to the FACS Lysing Solution package insert.
7. FACS brand flow cytometer. Refer to the appropriate instrument user's guide for information.

Procedure

Specimen Collection and Preparation

Collect blood aseptically by venipuncture^{1,2} into a sterile K3 EDTA VACUTAINER blood collection tube. Follow the collection tube manufacturer's guidelines for the minimum volume of blood to be collected. Store anticoagulated blood at room temperature (20° to 25°C) until ready for staining and lysing. Refer to the appropriate package insert for storage restrictions prior to staining.

WARNING: All biological specimens and materials with which they come into contact should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.



Lysing and Staining

1. Add 20 μL of fluorochrome-conjugated monoclonal antibody to 50 μL of whole blood in a 12 x 75-mm tube.
2. Vortex gently and incubate for 15 minutes in the dark at room temperature (20° to 25°C).
3. Add 450 μL of 1X FACS Lysing Solution.
4. Vortex gently and incubate for 15 to 30 minutes in the dark at room temperature.
5. Analyze on a FACS brand flow cytometer. Mix samples thoroughly before acquisition. Refer to the appropriate package insert for storage restrictions prior to analysis.

Recommendations

1. Use EDTA as the anticoagulant. BD has limited information concerning use of other anticoagulants such as heparin.
2. FACS Lysing Solution is specifically formulated for use with BD FACS brand flow cytometers.
3. Samples with nucleated red blood cells can show incomplete lysis of red blood cells because FACS Lysing Solution does not lyse nucleated erythrocytes. This can also occur when assaying blood samples from patients with certain hematologic disorders in which red cells are difficult to lyse, as in myelofibrosis, sickle-cell anemia, thalassemia, and spherocytosis.³
4. When using monoclonal antibodies that react with serum immunoglobulins, blood samples should be washed with 1X PBS or physiological saline prior to staining and lysing.⁴
5. A monoclonal antibody against a cell surface antigen or receptor that is shed into plasma (for example, IL-2 receptor) or occupied by plasma components (for example, complement receptors) can have reduced staining intensity when analyzed with lysed whole blood methodology.

References

1. Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes; Tentative Guideline. Villanova, PA: National Committee for Clinical Laboratory Standards; 1992. NCCLS document H42-T.
2. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture: Approved Standard. Villanova, PA: National Committee for Clinical Laboratory Standards; 1991. NCCLS document h2-A3.
3. Landay AL, Muirhead KA. Procedural guidelines for performing immunophenotyping by flow cytometry. Clin Immunol Immunopath. 1989;52:48-60.
4. Nicholson JKA, Rao PE, Calvelli T, et al. Artifactual staining of monoclonal antibodies in two-color combinations is due to an immunoglobulin in the serum and plasma. Cytometry. 1994;18:140-146.