Creighton UNIVERSITY

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

(Source: BD Technical Support Protocol, 2002)

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; then wash the sample to remove excess antibody and debris. Finally, analyze the cells by flow cytometry.

Equipment Required:

- K_3 EDTA VACUTAINER blood collection tubes (or equivalent).
- 12 x 75-mm capped polystyrene test tubes (or equivalent).
- Micropipettor with tips.
- Vortex mixer
- Centrifuge

Reagents:

- Fluorochrome-conjugated monoclonal antibodies to human cell surface antigens. Refer to the appropriate reagent package insert for more information.
- FACSLyse Solution (10X) (BD Cat. No. 349202).
- PBS4.
- FACSFix Solution.

Method:

1) Collect blood by venipuncture into a sterile K3 EDTA VACUTAINER blood collection tube. Store anticoagulated blood at room temperature (20° to 25°C) until ready for staining.
2) Add 50 µL of whole blood to a 12x75mm tube.
3) Add appropriate volume of fluorochrome-conjugated monoclonal antibody to the appropriate tube(s).
4) Vortex gently and incubate 15 to 30 minutes in the dark at room temperature (20° to 25°C).
5) Centrifuge at 500g for 5 minutes. Remove the supernatant.
6) Add 0.5 mL of FACSFix solution and mix thoroughly.
7) Store at 4°C until analyzed.

Notes:

1. Use EDTA as the anticoagulant.
2. Samples with nucleated red blood cells (such as pediatric samples) can show incomplete lysis of red blood cells because FACSLyse does not lyse nucleated erythrocytes.
3. 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. 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.