
Staining Cell Surface Targets for Flow Cytometry

Research Use Only

- [Protocol A: Cell Suspensions](#)
- [Protocol B: Human Lysed Whole Blood](#)

Introduction

Cell surface markers can be used to define cell subsets based on lineage and developmental stage, as well as function when they are labeled with fluorochrome-conjugated antibodies and analyzed by flow cytometry. These surface markers have different forms and functions, including receptors for both soluble and cell-bound ligands, ion channels, glycoproteins, phospholipids, and more. For example, CD4 is a surface marker for T helper cells that can be further differentiated based on expression of other chemokine receptors and cluster of differentiation (CD) markers. Live cells stained with antibodies can be sorted based on unique staining patterns and used for additional experiments.

General Notes

- *For optimal performance of fluorochrome conjugated antibodies, store vials at 2-8°C in the dark. Do not freeze.*
- *Prior to use, quick spin the antibody vial to recover the maximum volume. Vortexing the antibody vial is not recommended.*
- *Except where noted in the protocol, all staining should be done on ice or at 2-8°C with minimal exposure to light.*
- *If storage of samples is necessary after staining with fluorochrome-conjugated antibodies, store the samples in IC Fixation Buffer (cat. no. [00-8222](#)) by combining 100 µL of sample with 100 µL of IC Fixation Buffer, or add 2 mL of 1-step Fix/Lyse Solution (cat. no. [00-5333](#)). Samples can be stored in these buffers for up to 3 days in the dark at 2–8°C.*

NOTE: *There appears to be minimal impact on brightness of staining or FRET efficiency/compensation of tandem dyes, such as APC-eFluor® 780 or PE-Cyanine7, when using the IC Fixation Buffer (cat. no. [00-8222](#)) or 1-step Fix/Lyse Solution (cat. no. [00-5333](#)) for storage of samples. Differences in fixation buffer quality can affect fluorochrome brightness or FRET efficiency. Some generalizations regarding fluorophore performance after fixation can be made, but clone-specific performance should be determined empirically.*

NOTE: *Staining with Fixable Viability Dyes (FVD) is recommended before fixing samples to allow for gating on live cells during analysis by flow cytometry.*

For additional questions, please contact Technical Support at +1-888-810-6168 (US) or +43 1 796 4040 120 (Europe/International), or send us an email at Tech_Support@affymetrix.com

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Protocol A: Cell Suspensions

Materials

- 12 x 75 mm round-bottom test tubes or 96-well U- or V-bottom microtiter plates
- Primary antibodies (directly conjugated or purified)
- Secondary reagents, if necessary (for indirect staining)
- Anti-Mouse CD16/32 Purified (cat. no. [14-0161](#)) or Human Fc Receptor Binding Inhibitor Purified (cat. no. [14-9161](#))
- Flow Cytometry Staining Buffer (cat. no. [00-4222](#))
- [Optional] Viability solutions:
 - 7-AAD Viability Staining Solution (cat. no. [00-6993](#))
 - Propidium Iodide Staining Solution (cat. no. [00-6990](#))
 - Fixable Viability Dyes eFluor® 455UV (cat. no. [65-0868](#)), eFluor® 450 (cat. no. [65-0863](#)), eFluor® 506 (cat. no. [65-0866](#)), eFluor® 660 (cat. no. [65-0864](#)) and eFluor® 780 (cat. no. [65-0865](#))

Experimental Procedure

NOTE: *Antibody-binding kinetics are temperature-dependent. Staining on ice may require longer incubation times. Furthermore, some antibodies may require non-standard incubation conditions that will be noted on the technical data sheet provided with the antibody.*

1. Prepare cells as described in "[Cell Preparation Protocols for Flow Cytometry](#)" found in our Best Protocols section.
2. [Optional] Block non-specific Fc-mediated interactions:
 - For mouse cells: Pre-incubate the cells with 0.5-1 µg of Anti-Mouse CD16/CD32 Purified per 100 µL for 10-20 minutes at 2-25°C before staining.
 - For human cells: Pre-incubate the cells with 20 µL of Human Fc Receptor Binding Inhibitor Purified per 100 µL for 10-20 minutes at 2-25°C before staining.
3. Aliquot 50 µL of cell suspension (from 10⁵-10⁸) to each tube or well.
4. Combine the recommended quantity of each primary antibody in an appropriate volume of Flow Cytometry Staining Buffer so that the final staining volume is 100 µL (i.e. 50 µL of cell sample + 50 µL of antibody mix) and add to cells. Pulse vortex gently to mix.

NOTE: *Proceed to Step 8 for purified or biotinylated primary antibodies.*

5. Incubate for at least 30 minutes at 2-8°C or on ice. Protect from light.
6. Wash the cells by adding Flow Cytometry Staining Buffer. Use 2 mL/tube or 200 µL/well for microtiter plates. Centrifuge at 400-600 x g for 5 minutes at room temperature. Discard supernatant.
7. Repeat Step 6.

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NOTE: Proceed to Step 14 if all primary antibodies were directly conjugated to fluorochromes.

8. Incubate for 60 minutes at 2-8°C or on ice.
9. Wash the cells by adding Flow Cytometry Staining Buffer. Use 2 mL/tubes or 200 µL/well for microtiter plates. Centrifuge at 400-600 x g for 5 minutes at room temperature. Discard supernatant.
10. Repeat Step 9.
11. Dilute the appropriate fluorochrome-labeled secondary reagent in 100 µL of Flow Cytometry Staining Buffer and add to the cells. Incubate for at least 30 minutes at 2-8°C or on ice. Protect from light.
12. Wash the cells by adding Flow Cytometry Staining Buffer. Use 2 mL/tubes or 200 µL/well for microtiter plates. Centrifuge at 400-600 x g for 5 minutes at room temperature. Discard supernatant.
13. Repeat Step 12.
14. [Optional] Stain samples with a viability dye according to the appropriate "[Viability Dye Staining Protocols](#)" found in our Best Protocols section.
15. [Optional] For storage of samples before analysis, resuspend cells in 100 µL of Flow Cytometry Staining Buffer and add 100 µL of IC Fixation Buffer or 2 mL of 1-step Fix/Lyse Solution.
16. Resuspend cells in an appropriate volume of Flow Cytometry Staining Buffer.
17. Analyze samples by flow cytometry.

Protocol B: Human Lysed Whole Blood

Either the 10X RBC Lysis Buffer (Multi-species) or 1-step Fix/Lyse Solution (10X) may be used to lyse RBC after whole blood has been stained with fluorochrome-conjugated antibodies. Additionally, the 1-step Fix/Lyse Solution (10X) lyses RBC and fixes leukocytes in a single step. If lysed whole blood cells will be prepared using the 1-step Fix/Lyse Solution before staining, confirm that the antibodies in the staining panel recognize fixed epitopes on the antigens of interest. Please refer to the "Antibody Clone Performance Following Fixation/Permeabilization" table (page 60) for antibody clone performance following fixation/permeabilization.

Materials

- 10X RBC Lysis Buffer (Multi-species) (cat. no [00-4300](#)) or 1-step Fix/Lyse Solution (10X) (cat. no. [00-5333](#))
NOTE: Before using, the 10X RBC Lysis Buffer (Multi-species) and 1-step Fix/Lyse Solution (10X) must be diluted to 1X by adding 1 part buffer with 9 parts room temperature, reagent-grade water.
- 12 x 75 mm round-bottom test tubes
- Primary antibodies (directly conjugated or purified)

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- Secondary reagents, if necessary (for indirect staining)
- Human FC Receptor Binding Inhibitor Purified (cat. no. [14-9161](#))
- Flow Cytometry Staining Buffer (cat. no. [00-4222](#))
- [Optional] Viability solutions:
 - 7-AAD Viability Staining Solution (cat. no. [00-6993](#))
 - Propidium Iodide Staining Solution (cat. no. [00-6990](#))
 - Fixable Viability Dyes eFluor® 455UV (cat. no. [65-0868](#)), eFluor® 450 (cat. no. [65-0863](#)), eFluor® 506 (cat. no. [65-0866](#)), eFluor® 660 (cat. no. [65-0864](#)) and eFluor® 780 (cat. no. [65-0865](#))

Experimental Procedure

NOTE: *Antibody-binding kinetics are temperature-dependent. Staining on ice may require longer incubation times. Furthermore, some antibodies may require non-standard incubation conditions that will be noted on the technical data sheet provided with the antibody.*

1. Aliquot 100 μ L of whole blood to each tube.
2. [Optional] Block non-specific Fc receptor-mediated interactions with 20 μ L of Human Fc Receptor Binding Inhibitor Purified per 100 μ L of blood. Incubate for 10-20 minutes at 2-8°C or room temperature.
3. Combine the recommended quantity of each primary antibody in an appropriate volume of Flow Cytometry Staining Buffer so that the final volume of antibody mix is 50 μ L. Add to cells. Pulse vortex gently to mix.
4. Incubate for 20-30 minutes at room temperature. Protect from light.

NOTE: *Proceed to Step 7 if all primary antibodies were directly conjugated to fluorochromes.*

For purified or biotin conjugated antibodies:

5. Wash the cells by adding 2 mL of Flow Cytometry Staining Buffer. Centrifuge at 400–600 $\times g$ for 5 minutes at room temperature. Carefully discard supernatant.
6. Dilute the appropriate fluorochrome-labeled secondary reagent in 100 μ L of Flow Cytometry Staining Buffer and add to the cells. Incubate for 15–30 minutes at 2–8°C or on ice. Protect from light.
7. Without washing cells, add 2 mL of freshly prepared 1X RBC lysing solution and pulse vortex briefly. Incubate for 10–20 minutes at room temperature. Protect from light.

NOTE: Do not incubate longer than 20 minutes if using the 10X RBC Lysis Buffer (Multi-species) (cat. no. [00-4300](#)).

8. Centrifuge samples at 400-600 $\times g$ for 5 minutes at room temperature. Discard supernatant.
9. Add 2 mL of Flow Cytometry Staining Buffer and centrifuge at 400-660 $\times g$ for 5 minutes at room temperature. Discard supernatant.
10. Repeat Step 9.

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11. [Optional] For cells lysed using the 10X RBC Lysis Buffer, stain samples with a viability dye according to the appropriate “[Viability Dye Staining Protocols](#)” found in our Best Protocols section.

NOTE: Viability dyes, such as propidium iodide or 7-AAD, should not be used on cells lysed using the 1-step Fix/Lyse Solution as fixation can cause permeabilization of the cells. Fixable Viability Dyes (FVD) may be used to stain whole blood before using the 1-step Fix/Lyse Solution; refer to “[Viability Dye Staining Protocols](#)” found in our Best Protocols section for more details.

12. Resuspend cells in an appropriate volume of Flow Cytometry Staining Buffer.
13. Analyze samples by flow cytometry.

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