



Flow Cytometry Secondary Reagents

Goat F(ab')₂ Anti-mouse IgM-Phycoerythrin

Catalog Number: F0116

Lot Number: AAUH02

100 Tests

Intended Use

This reagent is designed for use as a secondary developing reagent in immunofluorescent assays, such as flow cytometry, where the primary antibody does not have a fluorescent reporter molecule, is of mouse origin and is of the IgM class.

Background Information

This polyclonal antibody preparation has been derived from goats immunized with mouse IgM (heavy and light chains). The goat IgG is first purified by affinity chromatography and then adsorbed to eliminate human cross reactivity. The IgG fraction is then digested with pepsin to generate F(ab')₂ fragments which have a reduced ability to interact with Fc receptors expressed on a variety of cells. The antibody is then conjugated to phycoerythrin (PE) for use in immunofluorescent type assays.

Reagents Provided

Goat F(ab')₂ Anti-mouse IgM (H+L chains)-PE:

Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Storage

Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Reagent Preparation

Goat anti-mouse IgM-Phycoerythrin is produced as the R-phycoerythrin derivative of F(ab')₂ fragments of goat IgG from animals immunized with mouse IgM. The reagent is provided in a ready-to-use liquid format containing phosphate buffered saline with 0.5% BSA and 0.1% NaN₃ as preservatives. R-Phycoerythrin has an absorption spectrum from 480 - 566 nm and has optimal emission at 575 nm. Store reagent at 2° - 8° C. **DO NOT FREEZE**. Dispose of azide containing liquids with caution and according to local regulations.

Sample Staining

1. Cells of interest (1 - 5 x 10⁵ cells) are stained with a mouse IgM primary antibody according to the antibody manufacturer's instructions.
2. After the recommended incubation period, the cells are washed 3 times with a PBS buffer followed by centrifugation at 250 x g for 5 minutes.
3. The cell pellet is resuspended in up to 200 µL of PBS and 10 µL of goat anti mouse IgM-PE is added to each reaction.
4. The cells are incubated for 30 minutes at 2° - 8° C in the dark and then washed 3 times as indicated in step # 2.
5. The cell pellet is resuspended in 400 µL of PBS for analysis by flow cytometry.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.