Flow Cytometry Secondary Reagents

Goat F(ab')$_2$ Anti-mouse IgM-Fluorescein

Catalog Number: F0118

Lot Number: AAUG01

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 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 carboxyfluorescein (CFS) for use in immunofluorescent type assays.

Reagents Provided

Goat F(ab')$_2$ Anti-mouse IgM (H+L chains)-CFS: 1 mL of goat anti-mouse IgM-CFS at a concentration of 25 μg/mL in phosphate buffered saline containing 0.5% BSA and 0.1% azide as preservatives.

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-Carboxyfluorescein (CFS) is produced as the CFS 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% NaN_3 as preservatives. CFS has an absorption maximum of 490 nm and has emission maximum of 520 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 10^5 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-CFS 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.

R&D Systems, Inc.
1-800-343-7475

3/09