IGG-PZF2001

Antibody Internalization Detection Reagent

Pack Size: 100 tests/500 tests

Catalog Number： IGG-PZF2001

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedure

PRODUCT DESCRIPTION

Antibody Internalization Detection Reagent (Cat. No. IGG-PZF2001) is formed by the reagent with a pH-sensitive red fluorescent dye. It can be used to assess the endocytosis of antibodies by labeling the human IgG Fc region. After co-incubation with the target antibody, a stable fluorescent complex can be formed to detect the endocytosis of the antibody in the cells. Furthermore, with the pH-sensitive dye, not only can it significantly enhance the fluorescence signal in an acidic environment but also prevent the background noise.

SPECIFICITY

Human IgG Fc Region

LABEL TYPE

pH-Sensitive Dye

Excitation Wavelength: 643 nm

Emission Wavelength: 660 nm

STORAGE

For long-term storage, the product should be stored at lyophilized state at -20°C or lower.

Please protect from light and avoid repeated freeze-thaw cycles.

This product is stable after storage at:

-20°C to -70°C for 24 months in lyophilized state.

-20°C to -70°C for 12 months after reconstitution.

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

REQUIRED REAGENTS/EQUIPMENT NOT SUPPLIED

Cell Culture Medium

FACS Buffer

Whole IgG Primary Antibodies

Suspension or Adherent Cell

96-well Plates

CO_2 Incubator

Centrifuge

Instruments (Flow Cytometer, Fluorescence microscope)

PREPARE ANTIBODY INTERNALIZATION DETECTION REAGENT STOCK SOLUTION

Reconstitute the lyophilized Antibody Internalization Detection Reagent with 50ul(100tests)/250ul(500tests) sterile deionized water to a stock solution. Solubilize for 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking or vortexing.

PROTOCOL FOR FACS OF SUSPENSION CELL

1. Prepare sufficient volume of 4X working solution (8 μg/ml) of primary antibody in cell culture medium and add 25 μl of 4X antibody working antibody solution to each well of a 96-well plate.
2. Dilute the stock solution 50 times to prepare 4X working solution (4 μg/ml) of Antibody Internalization Detection Reagent and add 25 μl of 4X reagent working solution to each well of the 96-well plate from Step 1. Incubate at room temperature for 10 minutes to allow the labeling complexes to form.
3. Prepare suspension cells at 2×10^6 cells/mL in cell culture medium.
4. Add 50 μL of cells to each well of the 96-well plate containing the labeling complexes (from Step 2). Incubate at 37°C for 1-4 hours.
5. Harvest the cells, wash the cells 3 times by FACS buffer and resuspend the cells in 200 μL PBS.
6. Transfer the cell suspension into flow tube and detect the cells by Flow cytometry.

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

PROTOCOL FOR FACS OF ADHERENT CELL

1. Prepare sufficient volume of 4X working solution (8 μg/ml) of primary antibody in cell culture medium and add 25 μl of 4X primary working antibody solution to each well of a 96-well plate.
2. Dilute the stock solution 50 times to prepare 4X working solution (4 μg/ml) of Antibody Internalization Detection Reagent and add 25 μl of 4X reagent working solution to each well of the 96-well plate from Step 1. Incubate at room temperature for 10 minutes to allow the labeling complexes to form.
3. Prepare suspension cells at 2×10^6 cells/mL in cell culture medium.
4. Add 50 μL of cells to each well of the 96-well plate containing the labeling complexes (from Step 2). Incubate at 37°C for 1-4 hours.
5. Harvest the cells, wash the cells 3 times by FACS buffer and resuspend the cells in 200 μL PBS.
6. Transfer the cell suspension into flow tube and detect the cells by Flow cytometry.

PROTOCOL FOR FLUORESCENCE IMAGING OF ADHERENT CELL

1. Harvest the adherent cells and plate cells at a density of 5000-10000 cells/well in 96-well culture plates. Incubate cells at 37°C with 5% CO_2. After the cells adhere, adjust volume so that each well contains 50 μL of culture medium.
2. Prepare sufficient volume of 4X working solution (8 μg/ml) of primary antibody in cell culture medium and add 50 μl of 4X primary working antibody solution to 1.5 ml centrifuge tube or other suitable containers.
3. Dilute the stock solution 50 times to prepare 4X working solution (4 μg/ml) of Antibody Internalization Detection Reagent and add 50 μl of 4X reagent working solution to 1.5 ml centrifuge tube from Step 2. Incubate at room temperature for 10 minutes to allow the labeling complexes to form.
4. Add 50 μL of the labeling complex (from Step 3) to each well of the 96-well plate (from Step 1). Incubate at 37°C with 5% CO_2 for 2-24 hours.
5. Remove the supernatants and 100 μl of PBS to each well of the 96-well plate..
6. Analyze cells using fluorescence microscopy or high content analysis.

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

OPTIMIZATION

1. The protocol described here is for performing antibody binding assays with one 96-well plate of antibodies. The recommended dosage is the result of tests conducted on specific cell lines. To meet your detection requirements, it is recommended that you conduct certain conditional explorations based on the dosage we recommend.
2. If you have a high detection background, please add the following steps:
   （1） Co-incubate the cells and the labeling complexes at 4°C for 1 hour in Step 4.
   （2） Aspirate the supernatant, then gently wash the cells three times with 1×PBS.
   （3） Add 100 µl of assay medium to each well of the 96-well plate and incubate cells at 37℃ with 5% CO_2 for appropriate time.
   （4） Analyze cells by Flow cytometry or fluorescence microscopy.

US and Canada: **Tel**: +1 800-810-0816
Asia and Pacific: **Tel**: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com