pH Sensitive Anti-Human IgG Labeling Reagent

Pack Size: 20 tests/100 tests /500 tests

Catalog Number： IGG-ZFS307

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

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

PRODUCT DESCRIPTION

In in-vivo CAR research, the endocytic capacity of tLNP is a key factor for evaluation of the delivery efficiency. Our pH-sensitive Anti-Human IgG Labeling Reagent (Cat. IGG-ZFS307) specifically binds to the Fc region of human IgG antibodies, and the pH-sensitive dye can significantly enhance the fluorescence signal in an acidic environment. This enables FACS analysis to evaluate the endocytic efficiency of IgG-format antibody-conjugated LNPs.

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℃ 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 3 months after reconstitution.

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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 PH SENSITIVE ANTI-HUMAN IGG LABELING REAGENT STOCK SOLUTION

Reconstitute the lyophilized pH Sensitive Anti-Human IgG Labeling Reagent with

40 μL(20 tests)/200 μL(100 tests)/1000 μL(500 tests) sterile deionized water to a stock solution (50 μg/mL). 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 4 × working solution (8 μg/mLl) of primary antibody in cell culture medium and add 25 μL of 4 × antibody working solution to each well of a 96-well plate.

2. Dilute the stock solution 12.5 times to prepare 4 × working solution (4 μg/mL) of pH Sensitive Anti-Human IgG Labeling Reagent and add 25 μL of 4 × 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℃ 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.

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PROTOCOL FOR ENDOCYTOSIS DETECTION OF tLNPs

Day 1:

Prepare Beads

Wash GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads before use.

1. Resuspend the Beads in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).

2. Transfer the desired volume of Beads to a tube.

3. Add an equal volume of PBS buffer containing 1% HSA, or at least 1 mL, and mix (vortex for 5 sec, or keep on a roller for at least 2 min).

4. Place the tube on a magnet for 2 min and then discard the supernatant.

5. Remove the tube from the magnet and resuspend the washed Beads in the same volume of T cell culture medium as the initial volume of Beads taken from the vial (step 2).

Prepare T cell and activate T cells

1. Prepare T cell culture medium, such as ImmunoCult™-XF T Cell Expansion Medium with 4 ng/mL IL-2.

2. Thaw and resuspended the $CD3^+$ T cells by T cell culture medium, and count the cells number and viability.

3. Start with 1×10^6 cells/mL $CD3^+$ T cells in 1 mL of T cell culture medium in a 12-well tissue culture plate.

4. Add 1 mL pre-washed and resuspended GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads to obtain a Beads-to-cells ratio of 1:1.

5. Mix well and incubate the 12-well-plate in the CO_2 incubator for 48 hours.

Day 3:

Harvest the activated T cells and use directly for further analysis.

1. For flow cytometry applications, remove the Beads prior to staining. Place the tube on a magnet for 2 min to separate the Beads from the solution. Transfer the supernatant containing the cells to a new tube.

2. Wash the cells twice by ImmunoCult™-XF T Cell Expansion Medium and add 100 µL ImmunoCult™-XF T Cell Expansion Medium containing 1×10^6/mL $CD3^+$ T cells into 96-well plate per well.

3. Dilute pH Sensitive Anti-Human IgG Labeling Reagent (Cat. No. IGG-ZFS307) with ImmunoCult™-XF T Cell Expansion Medium to 50 µL series of working solution, then add an equal volume of ImmunoCult™-XF T Cell Expansion Medium containing tLNP-hCD8 Ab or isotype control, final volume of 100 µL, incubate at 37 ℃ for 15-

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30 minutes to allow the labeling complexes to form.

4. Add the labeling complexes (from Step 3) into the cells, mix well and incubate in the CO_2 incubator for 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.

7. Analyze the result data using FCS Express 7Plus and GraphPad Prism 5 software.

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