Protocol for Detection of Antibody Internalization

1. Incubate pH Sensitive IgG Labeling Reagent with Antibody

1.1 Dissolve pH Sensitive IgG labeling reagent (22310) in freeze-dried powder according to COA using deionized water.

1.2 Mix antibody with the pH Sensitive IgG labeling reagent (22310) in 9:1 ratio by weigh (2:1 ratio by molarity), and then dilute the mixture of the antibody and pH Sensitive IgG labeling reagent to two-fold of a designed concentration using complete culture medium.

1.3 Incubate at room temperature in dark for 1 hour to obtain antibody-pH Sensitive IgG labeling reagent (22310) complex.

2. Incubate the Complex of Antibody-pH Sensitive IgG Labeling Reagent with Cells

2.1 Collect, wash cells and adjust cell concentration using complete culture medium ($1\text{-}2\times10^5$ cells/mL for suspend cells or $0.5\text{-}1\times10^5$ cells/mL for adherent cells). Add 100 µL cell suspension to each hole of the 96 well plate.

2.2 Add 100 µL Antibody-pH Sensitive IgG labeling reagent (22310) complex to each hole. Then incubate in a 5% CO2 incubator at 37 ℃.

3. Detect the Internalization with Flow Cytometry

After incubation for 18-24 hours, the internalization effect of the antibody was detected by flow cytometry using FITC or AF488 channels.

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