DiTag™ pH sensitive IgG labeling reagents

Catalog No: AME100001

DiTag ™ pH sensitive IgG labeling reagents provide an easy solution to measure internalization activities of antibodies. This reagent utilizes a pH-sensitive fluorescently labeled Fc binding protein that binds to IgG antibodies from various species, resulting in the formation of a fluorescently labeled antibody-reagent complex. After antibody internalization, the surrounding pH becomes acidic and significantly enhances fluorescence signal of antibody-reagent complex. The fluorescence intensity can be used as an indicator to determine the internalization activity of antibodies. By measuring the strength of the fluorescence signal, researchers can assess the efficiency of antibody internalization into cells. This information is crucial in understanding the cellular uptake mechanism of antibodies and assessing their efficacy in targeted therapies or diagnostic applications. Additionally, monitoring the fluorescence intensity can also provide insights into the kinetics of antibody internalization, helping researchers optimize experimental condition and improve the design of antibody-based drug delivery systems.

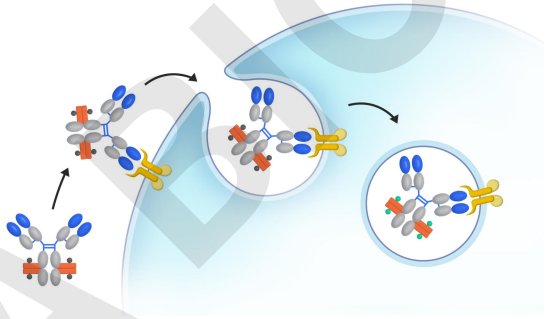

Table 1 Contents and storage

Description	DiTag™ pH sensitive IgG labeling reagent
Applications	Antibody internalization labeling
Formulation	Lyophilized from 1xPBS (pH 7.4). Normally 5% - 8% trehalose is added as protectants before lyophilization. Please see Certificate of Analysis for specific instructions.
Storage	The reagents are supplied in lyophilized form. We recommend storing the vial(s) at -20 °C, desiccated and protected from light. Once reconstituted, the reagents can be stored at 2-8°C for 1~2 weeks, or with 50% glycerol at -20°C.
Excitation/emission maxima	505/525 nm
Detection method	Flow cytometry, detected with FITC or AF488 filter
MW	The product has a MW of 33.4 kDa

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Protocol : DiTag™ pH sensitive IgG labeling reagents

1.Reconstitution of AME100001

Briefly centrifuge or tap down the vial of AME100001 to ensure that all product is collected at the bottom of the vial. Add the correct amount of sterile ddH2O to the vial to make the final concentration same as what indicated on COA. Reconstitute AME100001 with gentle agitation.

2.Prepare antibody-AME100001 complex in 2× working solution

Before actual internalization cell assay, a 2× working solution for Antibody -AME100001 complex needs to be prepared first. For the best results, we recommend test antibody to mix with AME100001 at 2:1 in molar ratio (9:1 by mass calculation).

Based on the final experimental setting, calculate the desired amount of test antibody and AME100001 stock solution (prepared in step1), mix thoroughly and add enough complete culture medium to make 100ul in final volume. Leave the 2× antibody-AME100001 complex solution at room temperature for 1hr and protected from light exposure by covering with aluminum foil.

3.Internalization Assay

Prepare the cells at 1~ 2 × 10^5 cells/mL in cell culture medium.

Add 100ul of cells to each well of a 96-well plate containing 100ul of 2X antibody-AME100001 working solution from step 2.

Incubate the cells with antibody-AME100001 complex at 37°C for 18-24hours under standard cell culture conditions.

The internalization efficiency of antibodies was measured using flow cytometry.

Assay Data : DiTag™ pH sensitive IgG labeling reagents

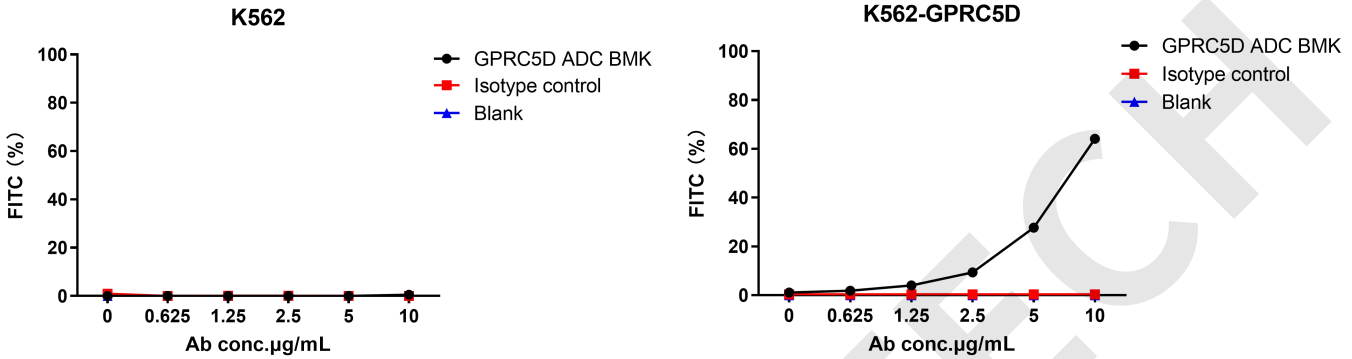

Figure1. The fluorescent signal from GPRC5D ADC BMK-AME100001 conjugate is only detected in GPRC5D positive cells (K562-GPRC5D stable expression cell line), indicating specific internalization.

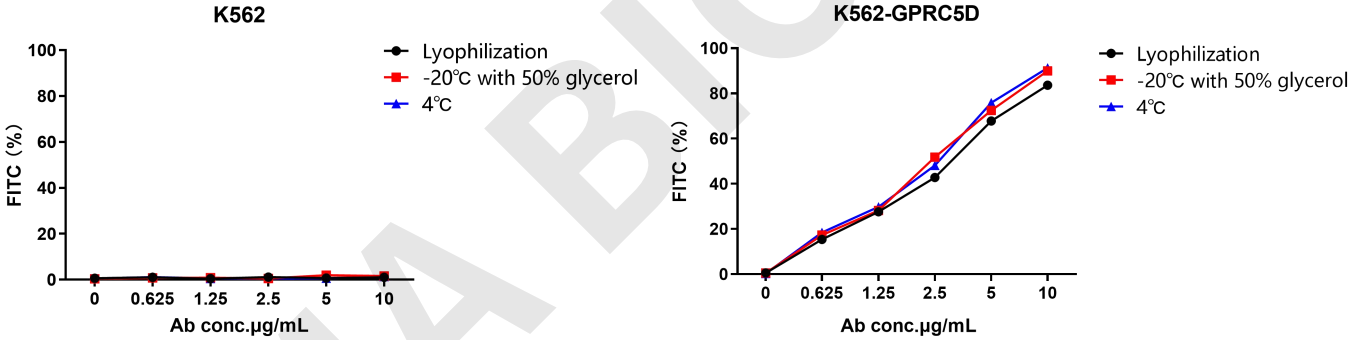

Figure2. Stability test of AME100001. Three storage methods are tested: lyophilization and reconstitution, liquid with 50% glycerol at -20°C, liquid at 4°C. All three methods exhibit excellent stability.

Ordering information

Cat. No.	Product name	Unit size
AME100001-10ug	DiTag™ pH sensitive IgG labeling reagent	10ug
AME100001-50ug	DiTag™ pH sensitive IgG labeling reagent	50ug
AME100001-100ug	DiTag™ pH sensitive IgG labeling reagent	100ug

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FAQ

1. **What is the MW of Fc binding protein in DiTag™ pH sensitive IgG labeling reagent?**
   The MW of Fc binding protein in DiTag™ pH sensitive IgG labeling reagents is 33.4kDa.
2. **How much antibody and cells should I use with this reagent?**
   When labeling an antibody with DiTagTM pH sensitive IgG labeling reagent, we suggest a molar ratio of 1:2 reagent: antibody in the reaction. For example, if you are labeling a 150kDa candidate antibody with DiTagTM reagent, you can use 1ug of reagent for 9ug of antibody and test antibody internalization using 5000-10000 cells. To determine the optimal conditions for your antibody candidates and cells, you should try different ratios and number of cells.
3. **Why is fixation not recommended for cells after incubation of antibody-reagent complex?**
   The pH-sensitive dye used in reagent in relatively non-fluorescent until it enters the acidic endosome, at which point its fluorescence increases. They should only be used on live cells to detect pH of cellular compartments. Upon fixation of the cells, the cell membrane is compromised and you are only measuring the pH of the buffer the samples are held in. For this reason, we do not recommend fixing samples.
4. **What is the fluorescence excitation/emission maxima for the pH-sensitive dye, provided in the reagent?**
   The fluorescence excitation/emission maxima for the pH-sensitive dye is 505/525 nm.
5. **Which IgG subtypes can we use with this reagent?**
   The DiTagTM pH sensitive IgG labeling reagents can be used for human IgG1、IgG2 and IgG4, rabbit IgG, mouse IgG2a and IgG2b.
6. **Are there any specific requirements for the downstream applications? Can we use flow cytometry and/or Incucyte after labeling and antibody internalization?**
   We test antibody internalization using flow cytometry. Live cell dynamic imaging and analysis system such as Incucyte has not been tested.
7. **How to reconstitute the reagent? Can we apply the antibody-reagent complex directly to the cells? Is it sterile?**
   Deionized water is recommend for the reconstitution according to the COA. This reagent is not sterile, but wo have not observed cell contamination within 1-24 hours yet.
8. **Is this reagent stable? Does it need to be stored in aliquot? How long can we store this reagent?**
   The reagents are supplied in lyophilized form. We recommend storing the vial(s) at -20 °C , desiccated and protected from light. Once reconstituted, the reagents can be stored at 2-8°C for 1~2 weeks, or with 50% glycerol at -20°C.

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