DiTag™ pH Sensitive IgG Labeling Reagent Plus

Introduction

DiTag™ pH Sensitive IgG Labeling Reagent Plus (**Catalog No. AME100002**) is a specialized reagent designed for evaluating antibody-drug conjugate (ADC) candidate internalization using a pH-sensitive dye. The reagent is compatible with human IgG1, IgG2, IgG3, IgG4, rabbit IgG, and mouse IgG1, IgG2a, IgG2b, and IgG3. It is supplied as a lyophilized powder and must be reconstituted before use.

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1. Reagent Preparation

1.1 Reconstitution of AME100002

- Centrifuge the lyophilized sample at 5000g for 3-5 minutes at room temperature to ensure the sample settles at the bottom of the tube.
- Dissolve the sample in sterile, deionized water (ddH_2O) to the same concentration as before lyophilization.
- After adding water, cover the tube and gently tap it 5-10 times, then pipette up and down to ensure complete dissolution. Note: Do not vortex or vigorously pipette the sample to avoid reagent degradation.
- Store the reconstituted reagent at 2-8°C for 1-2 weeks. For long-term storage, aliquot and store at -20°C with 50% glycerol.

1.2 Antibody Labeling with AME100002

- Mix the test antibody with AME100002 at a suggested starting **mass ratio of 3.5:1** (equivalent to a 1:2 molar ratio).

  Note: For optimal results, adjust the antibody-to-AME100002 ratio based on the specific antibody and membrane protein on the cell being used for the internalization test. For example, if the antibody affinity is low or the internalization signal is weak, increase the amount of AME100002; if the affinity or internalization signal is high, reduce the amount of reagent.

- Dilute the antibody-AME100002 mixture in complete culture medium to prepare a **2X working solution.** The antibody concentration in this solution should be 2X the final testing concentration.
- Incubate in the dark at **room temperature for 1 hour** to form the Ab-AME100002 complex.

2. Cell Incubation with Labeled Antibody

2.1 Cell Preparation

- Collect and wash cells twice with complete culture medium.
- Adjust cell concentration:
  - **Suspension cells**: 1 - 2 × 10^5 cells/mL.
  - **Adherent cells**: 0.5 - 1 × 10^5 cells/mL.
- Plate 100 µL of cell suspension per well in a 96-well plate.

2.2 Antibody Incubation with Cells

- Add **100 µL of Ab-AME100002 complex (2X working solution)** per well.
- Incubate at **37°C in a 5% CO_2 incubator** for 18-24 hours.

3. Flow Cytometry Analysis

- Analyze antibody internalization using **FITC or AF488 channels**.
- Acquire at least **2,000 events per sample** for accurate quantification.
- Compare fluorescence intensity shifts between control and treated samples.

4. Data Interpretation and Troubleshooting

4.1 Expected Results

- Increased fluorescence signal indicates antibody internalization into acidic compartments.
- Lower fluorescence suggests poor internalization or inadequate labeling.

4.2 Troubleshooting Tips

Issue	Possible Cause	Solution
Weak or No Signal	Insufficient labeling (e.g., low reagent concentration)	Increase incubation time or reagent amount to enhance signal strength.
	Antigen density on the cell surface is too low to detect interaction efficiently	Optimize antigen expression (e.g., transfection or protein expression strategies).
	Low affinity antibody	Test antibodies with varying affinities for the optimal signal-to-noise ratio.
	Antigen internalization is slow or incomplete	Adjust experiment conditions for optimal uptake
	The antibody enters the lysosomal degradation pathway	Investigate lysosomal degradation.
	Incorrect experimental setup or lack of proper controls	Ensure positive and negative controls are included to validate assay setup and antibody performance.
High background	Non-specific binding (e.g., lack of proper blocking or excessive antibody concentration)	Increase washing steps, optimize antibody concentration, or try different blocking reagents to reduce non-specific binding.
Inconsistent results	Cell viability issues (poor health of cells used in assay)	Ensure healthy and properly maintained cell cultures (check confluence, passage number, etc.).
	Variability in antibody quality or batch-to-batch inconsistency	Standardize antibody preparation or check antibody lot consistency for reproducibility.

5. Storage and Stability

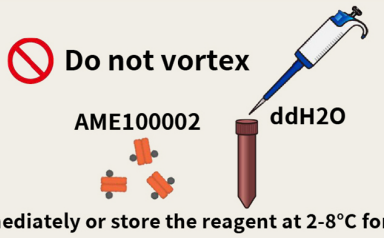
- The reagents are supplied in lyophilized form. We recommend storing the vial(s) at **-20°C**, desiccated and protected from light. Once reconstituted, the reagents can be stored at **2-8°C for 1-2 weeks**, or with **50% glycerol at -20°C**.
- Avoid repeated freeze-thaw cycles.

For further details, please refer to the product datasheet or contact **DIMA Biotech technical support** (info@dimabio.com).

Step-by-Step Protocol

Reconstitution of AME100002

- Centrifuge at 5000g for 3-5 minutes at RT.
- Dissolve in sterile ddH_2O to the same concentration as before lyophilization.



Use immediately or store the reagent at 2-8°C for 1 week.

Antibody Labeling

Incubate in the dark at RT for 1 hour

- Mix the test antibody with AME100002 at a mass ratio of 3.5:1 (1:2 molar ratio).
- Dilute the mixture to prepare a 2X working solution

Cell Preparation

- Collect and wash cells twice.
- Adjust cell concentration:
  - Suspension cells: 1-2 × 10^5 cells/mL.
  - Adherent cells: 0.5-1 × 10^5 cells/mL.

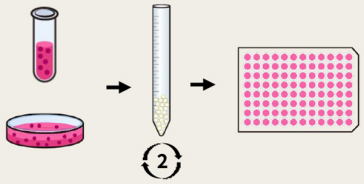
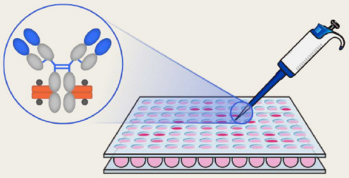


Plate 100 µL of cell suspension per well

Antibody Incubation with Cells

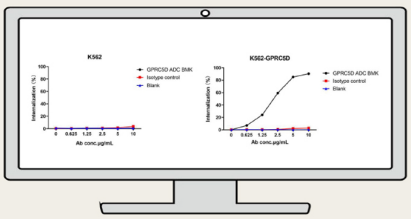


Incubate at 37°C in a 5% CO_2 incubator for 18-24 hours.

Add 100 µL of Ab-AME100002 complex (2X working solution) per well.

Flow Cytometry Analysis

- Analyze antibody internalization using FITC or AF488 channels.
- Acquire at least 2,000 events per sample for accurate quantification.



Data Interpretation

- Increased fluorescence signal indicates antibody internalization.
- Lower fluorescence suggests poor internalization or inadequate labeling.