

Antibody Internalization Detection

Standard Operating Procedure (SOP)

Cat. No.	Description
AME100003	DiTag™ MMAE IgG labeling reagent

1. AME100003 incubation with antibodies

- 1.1 Dissolve lyophilized AME100003 powder with ddH₂O according to COA.
- 1.2 Prepare a 4X working solution of the tested antibody in cell culture medium. To begin, a concentration of 1 µg/ml is recommended for testing purposes, thus resulting in a 4 µg/ml working solution.
- 1.3 Prepare a 4X working solution of AME100003 in cell culture medium. Maintain a concentration ratio of 9:1 with the tested antibody (2:1 molar ratio).
- 1.4 Mix equal volumes of the 4X working solutions of the tested antibody and AME100003 to achieve a 1:1 ratio. Incubate the mixture at 37°C for 1 hour to obtain the Ab-AME100003 complex at a 2X working solution concentration.

2. Incubation of Ab-AME100003 Complex with Cells

- 2.1 Collect and wash cells, adjusting the cell concentration using complete culture medium based on the growth rate of cells. A cell concentration of 1×10⁵ cells/mL is recommended. Add 50 µL of the cell suspension into each well of a 96 well plate.
- 2.2 Add 50 µL of the Ab-AME100003 complex (2X working solution) to each well. For adherent cells, add the complex after cell adhesion. Incubate the plate in a 5% CO₂ incubator at 37°C.

3. Detection of Killing Effect

After incubating for 2-3 days, assess the killing effect of the tested antibody using the CCK8 or CTG method following the manufacturer's protocols.