

Antibody Internalization Detection

Standard Operating Procedure (SOP)

Cat. No.	Description
AME100005	DiTag™ Eribulin IgG labeling reagent

1. AME100005 incubation with antibodies

- 1.1 Dissolve lyophilized AME100005 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 1µg/ml is recommended for testing purposes, thus resulting in a 4µg/ml working solution.
- 1.3 Prepare a 4X working solution of AME100005 in cell culture medium. The tested antibody and AME100005 were mixed at a mass ratio of 9:1 (molar ratio of 2:1).
- 1.4 Mix equal volumes of the 4X working solutions of the tested antibody and AME100005. Incubate the mixture at 37 °C for 1 hour to obtain the Ab-AME100005 complex at a 2X working solution concentration.

2. Incubation of Ab-AME100005 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 50µL of the cell suspension into each well of a 96 well plate.
- 2.2 Add 50µL of the Ab-AME100005 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.