

AME100003 for Detection of antibody internalization-SOP

1. AME100003 incubation with antibodies

- 1.1 Dissolve AME100003 in freeze-dried powder form according to COA using deionized water.
- 1.2 Prepare sufficient volume of 4X working solution (quadrupled the tested concentration) of tested antibody in cell culture medium. 1 μ g/ml maybe a good starting tested concentration for antibodies, so 4X working solution is 4 μ g/ml.
- 1.3 Prepare 4X working solution of AME100003 in cell culture medium. Concentration ratio of tested antibody with AME100003 is 9:1 (molar ratio 2:1).
- 1.4 Mix 4X working solution of tested antibody and 4X working solution of AME100003 at 1:1 volume ratio. Incubate at 37 $^{\circ}$ C for 1 hour to obtain Ab-AME100003 complex 2X working solution.

2. Ab-AME100003 complex incubation with cells

- 2.1 Collect and wash cells, adjust cell concentration using complete culture medium based on the growth rate of cells. 1 \times 10⁵ cells/mL maybe a proper concentration. Add 50 μ L cell suspension to each hole of the 96 well plate.
- 2.2 Add 50 μ L Ab-AME100001 complex 2X working solution to each hole. For adherent cells, add the complex after cell adhesion. Incubate in a 5% CO₂ incubator at 37 $^{\circ}$ C.

3. Killing effect detection

After incubation for 2-3 days, the killing effect of the tested antibody was detected by CCK8 or CTG method according to the manufacturer's standards.

