

# **AME100004 for Detection of antibody internalization-SOP**

## **1. AME100004 incubation with antibodies**

- 1.1 Dissolve AME100004 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. 1µg/ml maybe a good starting tested concentration for antibodies, so 4X working solution is 4µg/ml.
- 1.3 Prepare 4X working solution of AME100004 in cell culture medium. Concentration ratio of tested antibody with AME100004 is 1:1 (molar ratio 1:2).
- 1.4 Mix 4X working solution of tested antibody and 4X working solution of AME100004 at 1:1 volume ratio. Incubate at 37°C for 30 minutes to obtain Ab-AME100004 complex 2X working solution.

## **2. Ab- AME100004 complex incubation with cells**

- 2.1 Collect and wash cells, adjust cell concentration using complete culture medium based on the growth rate of cells.  $2 \times 10^5$  cells/mL maybe a proper concentration. Add 50µL cell suspension to each hole of the 96 well plate.
- 2.2 Add 50µL Ab- AME100004 complex 2X working solution to each hole. For adherent cells, add the complex after cell adhesion. Incubate in a 5% CO<sub>2</sub> incubator at 37°C.

## **3. Killing effect detection**

After incubation for 48-72h, the killing effect of the tested antibody was detected by CCK8 or CTG method according to the manufacturer's standards.