Signosis
Innovative Plate Assay Solutions

Beta Actin ELISA Kit

Catalog Number EA-6001 **(For Research Use Only)**

Introduction

Beta-Actin is highly conserved proteins and is ubiquitously expressed in all eukaryotic cells at a constant level regardless of experimental treatment or technical procedure in most cases. Therefore, measurement of beta-Actin is generally used as an internal control for experimental error. Signosis has provided b-actin sandwich ELISA assay specifically to detect the endogenous levels of beta-actin, which can be used for internal control for most of ELISA assays with human, mouse and rat samples.

Principle of the assay

Beta-actin ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes a rabbit polyclonal beta-actin capture antibody for immobilization on the microtiter wells, and a mouse monoclonal beta-actin detection antibody along with anti-mouse conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two antibodies, resulting in the beta-actin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of stop solution changing the color to yellow. The concentration of beta-actin is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

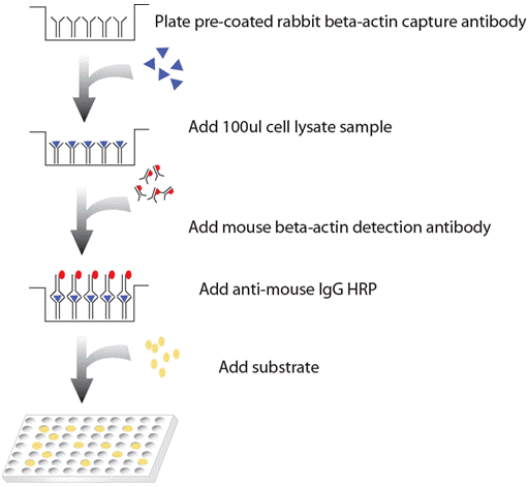

Diagram of ELISA

Materials provided with the kit

- 12x8 strip microplate coated with beta-actin capture antibody (4°C).
- Mouse beta-actin detection antibody (4°C).
- Anti-mouse IgG HRP (4°C)
- 1X Diluent buffer (4°C)
- 5X Assay wash buffer (4°C)
- Substrate (4°C)
- Stop Solution (4°C)

Material required but not provided

- Cell lysis buffer for ELISA (EA-0001)
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Sample preparation: Add 200ul 1X cell lysis buffer to one well of 12-well plate and pipetting up and down to detach the cells and incubate on ice for 5 minutes. Transfer the cell to 1.5ml tube and sonicate briefly on ice. The cell lysate was centrifuged for 5 minutes at 12000rpm. The supernatant is transferred to a new tube for downstream assay. If your sample is a well of 96-well plate (10^4 cells), you can add 100ul 1X Cell lysate buffer in the well and directly use the cell lysate for the assay.
- Dilute 200 times of mouse monoclonal beta-actin antibody with 1X Diluent buffer before use.
- Dilute 1000 times of anti-mouse HRP conjugate with 1X Diluent buffer before use.

Assay procedure

1. Cut the sealing film over the plate and remove it from the needed number of well strips. Make sure the rest of wells are well sealed.
2. Add 100 µl of sample per well and incubate at 37°C for two hours.
3. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process two times for a total of three washes. Complete removal of liquid at each wash by inverting the plate against a pile of clean paper towels.
4. Add 100µl of diluted mouse monclonal beta-actin detection antibody to each well and incubate for one hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µl of diluted anti-mouse IgG HRP conjugate to each well and incubate for one hour at room temperature.
7. Repeat the aspiration/wash as in step 3.
8. Add 100µl of substrate to each well and incubate for 15-30 minutes.
9. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 45 minutes.

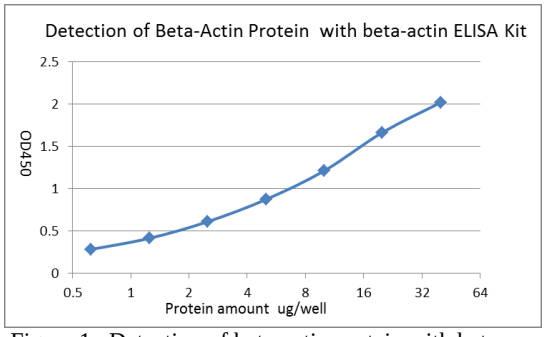

Figure 1. Detection of beta-actin protein with beta-actin ELISA kit. HeLa cells were grown in a 12-well plate. Cell lysate was prepared with Cell Lysis Buffer, 1:2 serially diluted with 1 x Diluent buffer and subject to ELISA assay.