SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com

Product Information

Cell-Based Protein Phosphorylation ELISA kits are very rapid, convenient and sensitive assay kits which can be used for measuring the relative amount of protein phosphorylation and monitoring the effect of various treatments, inhibitors (such as siRNA or chemicals), or activators in adherent cultured cells. Cell-Based ELISA kits are convenient and efficient for daily use.

Cell-Based Assay Procedure

NOTE: ALL incubations and wash steps must be performed under gentle rocking or rotation (~1-2 cycles/sec).

1. Design your experiment.

OPTIONAL: If seeding HUVECs, HMEC-1 or other loosely attached cells, coat the Uncoated 96-Well Microplate (ITEM A) by adding 100 µl poly-L-Lysine (Recommended Sigma-Aldrich®, Cat#: P4832) into each well and then follow manufacturer's instructions. A pre-coated CellBIND® microplate or other poly-lysine treated tissue culture plate may be used in place of ITEM A.

2. Seed 100 µL of 10,000 to 30,000 cells into each well of the Uncoated 96- Well Microplate (ITEM A) provided and incubate overnight at 37 °C with 5% CO_2.

NOTE: The optimal cell number used will vary on the cell line and the relative amount of protein phosphorylation. More or less cells may be used but this must be determined empirically.

The cells can be starved ~4–24 hours (depending on cell line) prior to treatment with inhibitors or activators.

3. Apply various treatments, inhibitors (such as siRNA or chemicals) or activators according to manufacturer's instructions and incubate for the desired time points.

NOTE: It is recommended to dissolve inhibitors or activators into serum-free cell culture medium before treating the cells (unless otherwise stated in the manufacturer's instructions.)

4. Discard the cell culture medium by flipping the microplate upside down and gently tapping the bottom of the microplate over a sink.

5. Wash by pipetting 200 µL of the prepared 1X Wash Buffer A (ITEM B) into each well. Discard the wash buffer (same as step 4) and wash 2 more times for a total of 3 washes using fresh wash buffer each time. After the final wash, gently blot the microplate onto a paper towel to remove any excess/remaining buffer.

NOTE: To avoid cell loss, do not pipette directly onto the cells. Instead, gently dispense the liquid down the wall of cell culture wells. Also avoid the use of vacuum suction or too forcefully tapping the microplate when discarding any solution.

6. Add 100 µL of Fixing Solution (ITEM D) into each well and incubate for 20 minutes at room temperature.

NOTE: The fixing solution is used to permeabilize the cells.

7. Repeat wash step 5.

8. Add 200 µL of prepared 1X Quenching Buffer (ITEM E) into each well and incubate 20 minutes at room temperature.

NOTE: The quenching buffer is used to minimize the background response.

9. Wash 4 times with 1X Wash Buffer A.

10. Add 200 µL of the prepared 1X Blocking Buffer (ITEM F) into each well and incubate for 1 hour at 37 °C.

11. Wash 3 times with the prepared 1X Wash Buffer B (ITEM C).

NOTE: If needed, the microplate may be stored at -80 °C for several days after this wash.

12. Add 50 µL of the prepared 1X primary antibody (ITEM G-1, G-2, G-3, H- 1, H-2 or H-3) into each corresponding well and incubate for 2 hours at room temperature.

13. Wash 4 times with 1X Wash Buffer B.

14. Add 50 µL of the prepared 1X HRP Conjugated secondary antibody (ITEM I-2) into each well and incubate for 1 hour at room temperature.

15. Repeat step 13.

16. Add 100 µL of the TMB Substrate (ITEM J) into each well and incubate for 30 minutes at room temperature in the dark.

17. Add 50 µL of the Stop Solution (ITEM K) into each well. Read at 450 nm immediately.

How It Works