Product Information

Phosphorylation ELISA Kits are a very rapid, convenient and sensitive assay kit that can monitor the activation or function of important biological pathways in cell lysates. By determining phosphorylated protein in your experimental model system, you can verify pathway activation in your cell lysates. You can simultaneously measure numerous different cell lysates without spending excess time.

Phosphorylation Assay Procedure

1. Bring all reagents to room temperature (18–25 °C) before use. It is recommended that all samples or Positive Control should be run at least in duplicate.

2. Add 100 µL of each sample or positive control into appropriate wells. Cover well with plate holder and incubate for 2.5 hours at room temperature or overnight at 4 °C with shaking.

3. Discard the solution and wash 4 times with 1× Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating. Invert the plate and blot it against clean paper towels.

4. Add 100 µl of prepared 1x biotinylated anti-phosphotyrosine antibody to each well. Incubate for 1 hour at room temperature with shaking.

5. Discard the solution. Repeat the wash as in step 3.

6. Add 100 µL of prepared 1X HRP-Streptavidin solution (see Reagent Preparation step 6) to each well. Incubate for 45 minutes at room temperature with shaking.

7. Discard the solution. Repeat the wash as in step 3.

8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with shaking.

9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately

How It Works

Capture Antibody pre-coated onto the 96-well plate

Biological samples and positive controls are added to the wells

Primary antibodies, anti-phosphorylated or anti-pan antibodies, are added to the wells

HRP-conjugated secondary antibody solution is added to the wells

OD=450 nm

Graph and Data Analysis

Phospho-HSP27 (S82) ELISA

Phospho-Hsp27 pan-Hsp27

Untreated

PMA

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