New Phosphorylation Assay Kits

PROTEIN PHOSPHORYLATION ASSAYS

Sigma-RBI is pleased to introduce a series of phosphospecific assay kits for the precise quantitation of phosphorylated or non-phosphorylated cell signaling proteins involved in phosphorylation cascades. Phosphospecific ELISAs offer researchers a sensitive, quantitative and economical alternative to traditional immunoblotting or functional assays.

Assay Platform

The format is a solid phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). A capture antibody specific for a protein (regardless of phosphorylation state) has been coated onto the wells of the microtiter plate. Antigen binds to the capture antibody. A detection antibody specific for the phosphorylated or non-phosphorylated protein is added and binds to the immobilized protein. An anti-rabbit IgG-HRP completes the four-member sandwich. The reaction is visualized by tetramethylbenzidene (TMB) substrate and the intensity of the color is directly proportional to the concentration of protein present in the original sample. The reaction is read at 450 nm in a microtiter plate reader.

Samples

Cell extracts are the samples of choice, because the proteins involved in the phosphorylation are present in intracellular compartments. Researchers may use the cell extraction procedure provided in the Technical Bulletin or utilize the **Sigma Mammalian Cell Lysis Kit** (Prod. No. **MCL-1**).

Advantages

Sensitive
- Picogram/mL quantities for non-phosphorylated ELISAs
- < 1.0 unit/mL for phosphorylated ELISAs
- 2 to 10 times more sensitive than traditional immunoblotting

Specific
- Highly specific for target protein and phosphorylation site
- Validated against immunoblotting performed with the same antigen and antibodies
- Validated by peptide competition

Quantitative
- Standard curve is run in each assay

Simple
- Precoated plates eliminate the coating step
- All incubations performed at room temperature
- Small sample size – only 10 µL (diluted to a total volume of 100 µL)
- Use any microtiter reader available on the market
- No additional equipment required

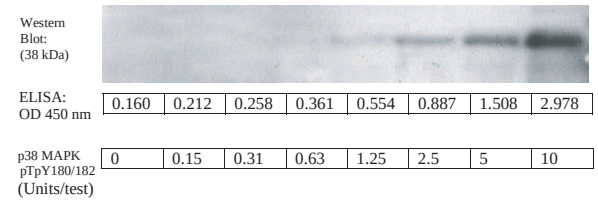
Stable
- Entire kit is stable in the refrigerator for 12 months
- Components are all stored at 2-8°C
- Stable liquid chromogen and stop reagents
- Two vials of standard extend the life-span of the kit

Fast
- Results in 4 hours

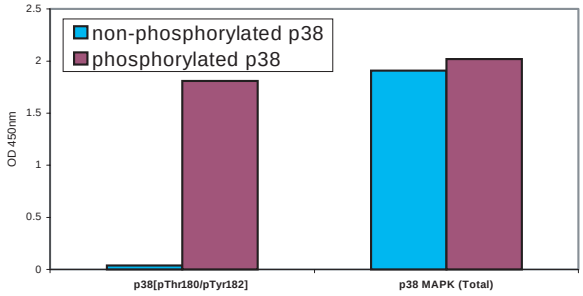
Economical
- Reagents may be used for multiple kit runs

Performance Characteristics
- Low intra- and inter-assay variability
- Linear standard curve
- Recovery ranges > 93%
- Each phosphospecific kit is normalized against non-phosphorylated ELISA

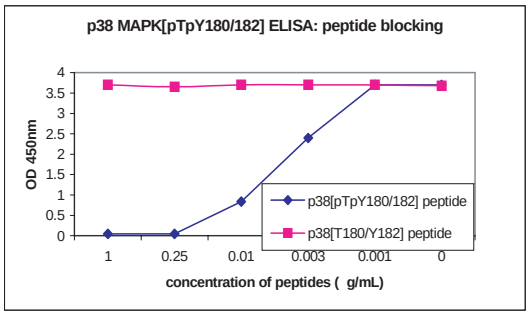
Sample Data using Sigma-RBI Phosphospecific ELISAs



Detection of p38 MAPK [pThr^{180}/pTyr^{182}] by ELISA vs. Immunoblotting. The sensitivity of this ELISA was compared to immunoblotting using known quantities of p38 MAPK (pThr^{180}/pTyr^{182}). The data presented show that the sensitivity of the ELISA is approximately 10X greater than that of immunoblotting. The bands shown in the immunoblotting data were developed using rabbit anti-p38 MAPK (pThr^{180}/pTyr^{182}), an alkaline phosphatase conjugated anti-rabbit IgG, followed by chemiluminescent substrate and autoradiography.



Recombinant p38 MAPK was phosphorylated using MKK6 enzyme *in vitro*. Non-phosphorylated p38 MAPK was used as control. The phosphorylated and non-phosphorylated p38 MAPK were analyzed with phospho-p38 MAPK (pThr^{180}/pTyr^{182}) ELISA and p38 MAPK ELISA. This phospho-p38 MAPK (pThr^{180}/pTyr^{182}) ELISA kit is specific for measurement of phosphorylated p38 MAPK at threonine 180 and tyrosine 182. The kit does not detect non-phosphorylated p38 MAPK protein.



Phospho-p38 MAPK (pThr^{180}/pTyr^{182}) ELISA Peptide Blocking. The specificity of this assay for p38 MAPK phosphorylated at threonine 180 and tyrosine 182 was confirmed by peptide competition. The data presented show that only the phosphorylated peptide containing phosphorylated threonine and tyrosine could block the ELISA signal. The same sequence containing non-phosphorylated threonine and tyrosine did not block the signal.

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New Phosphorylation Assay Kits

Akt/PKB ELISA
Prod. No. CS0160

Phospho-Akt/PKB (pThr^{308}) ELISA
Prod. No. CS0110

Phospho-Akt/PKB (pSer^{473}) ELISA
Prod. No. CS0120

Epidermal Growth Factor Receptor (EGFR) ELISA
Prod. No. CS0080

Phospho-Epidermal Growth Factor Receptor (EGFR) (pTyr^{1068}) ELISA
Prod. No. CS0150

Phospho-Epidermal Growth Factor Receptor (EFGR) (pTyr^{1173}) ELISA
Prod. No. CS0140

ERK 1&2 ELISA
Prod. No. EK0100

Phospho-ERK 1&2 (pThr^{185}/pTyr^{187}) ELISA
Prod. No. PE0100

Insulin Receptor β Subunit ELISA
Prod. No. CS0090

Phospho-Insulin Receptor β Subunit (pTyr^{1158}) ELISA
Prod. No. PI0100

Phospho-Insulin Receptor β Subunit (pTyr^{1162}/pTyr^{1163}) ELISA
Prod. No. PI0200

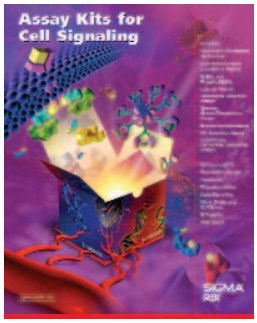
JNK 1&2 ELISA Human
Prod. No. CS0100

Phospho-JNK 1&2 (pThr^{183}/pTyr^{185}) ELISA
Prod. No. CS0130

p38 MAPK ELISA
Prod. No. PM0100

Phospho-p38 MAPK (pThr^{180}/pTyr^{182}) ELISA
Prod. No. CS0020

New Cell Signaling & Neuroscience Literature



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- Over 350 products are featured, including over 50 new products

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- 600 kits and reagents for apoptosis research are featured, including over 50 new products

Apoptosis Poster

- Over 300 products
- Numerous pathway illustrations

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