

p38 MAPK ELISA

p38 Mitogen Activated Protein Kinase ELISA

Product Number **PM0100**

Storage Temperature 2-8 °C

Product Information**Technical Bulletin****Product Description**

p38 MAPK ELISA is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) for quantitative detection of p38 MAPK protein in cell lysates. A monoclonal antibody specific for p38 MAPK (regardless of phosphorylation state) has been coated onto the wells of the multiwell strips provided. p38 MAPK standard dilutions, control specimens, and unknown samples are pipetted into these wells. During the first incubation, the p38 MAPK antigen binds to the immobilized (capture) antibody. After incubation, excess reagents are washed away and an antibody specific for total p38 MAPK is added to the wells. During the second incubation, this antibody serves as a detection antibody by binding to the immobilized p38 MAPK protein captured during the first incubation. After removal of excess detection antibody, a horseradish peroxidase-labeled anti-rabbit IgG (Anti-Rabbit IgG-HRP) is added. This binds to the detection antibody to complete the four-member sandwich. After a third incubation and washing to remove excess anti-rabbit IgG-HRP, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of p38 MAPK present in the original specimen. The optical density measured at 450 nm in the microplate reader is used to calculate the concentration of p38.

The ELISA detects p38 MAPK in human, monkey and mouse. This assay can be used to normalize the p38 MAPK content of the samples when using Phospho-p38 MAPK (pThr¹⁸⁰/pTyr¹⁸²) ELISA (Product No. CS0020).

p38 MAP kinase (MAPK), also known as a CDC-2-related protein kinase or CSBP (cytokine suppressive anti-inflammatory drug binding protein) is the human homolog of the *Saccharomyces cerevisiae* gene Hog1, which is a MAPK required for growth under high-osmolarity conditions. p38 MAPK is activated in response to a variety of extracellular stimuli including osmotic shock, inflammatory cytokines,

lipopolysaccharides (LPS), anisomycin, UV light and growth factors. The activation of p38 MAPK is mediated by several upstream kinases including MAP kinase kinase 3 (MKK3), MAP kinase kinase 6 (MKK6) and MAP kinase kinase 4 (MKK4, also known as SEK1 and JNKK1). These kinases phosphorylate p38 at threonine 180 and tyrosine 182 in the TGY motif, resulting in p38 activation. The p38 signaling transduction pathway plays an essential role in regulating many cellular processes including inflammation, cell differentiation, cell growth and death. Regulation of CDC25B phosphorylation by p38 is a critical event for initiating the G2/M checkpoint after ultraviolet radiation. Targets of p38 include transcription factors ATF-2, Max, MEF2C, CHOP, MAPKAPK2 (MAPK-activated protein kinase-2), and PRAK kinase (p38-related/ activated protein kinase).¹⁻⁵

Reagents

- **p38 MAPK Standard Human, 2 vials, Product No. P 8870** - purified full-length recombinant human p38 MAPK protein expressed in *E. coli*. Refer to vial label for quantity and reconstitution volume.
- **Standard Diluent Buffer, 25 mL, Product No. S 3943** - containing BSA and sodium azide as preservative. Ready to use.
- **Monoclonal Anti-p38 MAPK-coated 96 well plate, 1 plate, Product No. P 8995** - A plate using break-apart strips coated with monoclonal antibody specific for full-length p38 MAPK regardless of its phosphorylation state.
- **Anti-p38 MAPK, 11 mL, Product No. P 7620** - A detection antibody, produced in rabbit, which recognizes a full-length p38 MAPK regardless of its phosphorylation state. Ready to use.
- **Anti-Rabbit IgG-HRP Concentrate (100x), 1 vial, Product No. R 0403** - contains 50% glycerol, viscous. See *Reagent Preparation for handling, dilution and storage instructions*.
- **HRP Diluent, 25 mL, Product No H 8912** - contains thymol and BSA. Ready to use.

- **Wash Buffer Concentrate 25x, 100 mL, Product No. W 2639** - See *Reagent Preparation for handling, dilution and storage instructions*.
- **Stabilized Chromogen (TMB), 25 mL, Product No. S 3318** – Light sensitive. Ready to use.
- **Stop Solution, 25 mL, Product No. S 2818** – Ready to use.
- **Plate Covers, Adhesive strips, 3 each, Product No. P 4870**

10% Glycerol
 0.1% SDS
 0.5% Deoxycholate
 1 mM PMSF (stock is 0.3 M in DMSO)
PMSF is very unstable and must be added prior to use, even if added previously.
 Protease inhibitor cocktail (Product No. P 2714)
 Add 250 µL of reconstituted cocktail per 5 mL of Cell Extraction Buffer.

Reagents and Equipment required but not provided

- Multiwell plate reader capable of readings at 450 nm
- Calibrated adjustable precision pipettes for volumes between 5 µL and 1,000 µL.
- Cell extraction buffer (see recommended extraction procedure)
- Deionized or distilled water.
- Plate washer (optional), use squirt bottle, manifold dispenser, etc.
- Graph paper: linear, log-log, or semi-log, as desired
- Glass or plastic 1.0–1.5 mL tubes for diluting and aliquoting standard
- Absorbent paper towels to blot the plate
- Calibrated beakers and graduated cylinders in various sizes
- Vortex mixer

Stable for 2-3 weeks at 4 °C or for up to 6 months when aliquoted (without protease inhibitors and PMSF added) and stored at –20 °C.

Thaw on ice. Add the protease inhibitors just before use.

Procedure for Extraction of Proteins from Cells

The recommended Cell Extraction Buffer and procedure are optimized to achieve effective protein phosphorylation. Researchers may use the procedures that work best in their hands. In such case, they will have to assay lysates for the satisfactory extraction and/or phosphorylation.

Protocol for Cell Extraction

1. Collect cells in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).
2. Wash twice with cold PBS.
3. Remove and discard the supernatant and collect the cell pellet. (At this point the cell pellet can be frozen at –70 °C and lysed at a later date).
4. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes on ice with vortexing at 10-minute intervals. The volume of Cell Extraction Buffer depends on the cell number in cell pellet and expression of p38 MAPK. For example, 10⁸ Jurkat cells grown in RPMI plus 10% FBS can be extracted in 1 mL of Extraction Buffer.
5. Transfer extracts to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4 °C.
6. Aliquot the clear lysate to clean microcentrifuge tubes.

Precautions and Disclaimer

The kit is for R&D use only, not for drug, household or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

- Samples of choice – extracts of cell lysates
- Samples should be frozen if not analyzed shortly after collection.
- Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
 - Cell Extraction Buffer
 - 10 mM Tris, pH 7.4
 - 100 mM NaCl
 - 1 mM EDTA
 - 1 mM EGTA
 - 1 mM NaF
 - 20 mM Na₄P₂O₇
 - 2 mM Na₃VO₄
 - 1% Triton X-100

Before assay: extracted cell lysate samples containing p38 MAPK protein should be diluted with Standard Diluent Buffer at least 1:10. This dilution is necessary to reduce the matrix effect of the cell lysate buffer. Example: 0.1-1 µL of the clarified cell extract diluted to a volume of 100 µL/well in Standard Diluent Buffer is sufficient for the detection of p38 MAPK.

Reagent Preparation

p38 MAPK Standard

1. Reconstitute one vial of Standard with Standard Diluent Buffer according to label directions. Mix gently and wait 10 minutes to ensure complete reconstitution. Label as **2000 pg/mL p38 MAPK**.
2. Prepare serial standard dilutions as follows:

Tube#	Standard Buffer	Standard from tube #:	Final p38 pg/mL
1	Reconstitute according to label instructions		2000 pg/mL
2	0.25 mL	0.25 mL (1)	1000
3	0.25 mL	0.25 mL (2)	500
4	0.25 mL	0.25 mL (3)	250
5	0.25 mL	0.25 mL (4)	125
6	0.25 mL	0.25 mL (5)	62.5
7	0.25 mL	0.25 mL (6)	31.2
8	0.5 mL	-	0

Mix thoroughly between steps.

3. Use within 1 hour of reconstitution.
Reconstituted Standard can be frozen at -70°C and thawed one time only without loss of immunoreactivity.

Anti-Rabbit IgG-HRP, 100X Concentrate

Contains 50% glycerol and is very viscous. To ensure accurate dilution follow instructions below:

1. Equilibrate to room temperature, mix gently, pipette slowly.
2. Remove excess concentrate solution from pipette tip with clean absorbent paper.
3. Mix: 10 μl IgG-HRP concentrate +1 mL HRP Diluent (sufficient for one 8-well strip, prepare more if needed)
4. Label as **Anti-Rabbit IgG-HRP Working Solution**.
5. Return the unused Anti-Rabbit IgG-HRP concentrate to the refrigerator.

Wash Buffer

1. Equilibrate to room temperature and mix to redissolve any precipitated salts.
2. Mix 1 volume Wash Buffer Concentrate 25X + 24 volumes of deionized water
3. Label as **Working Wash Buffer**.
4. Store both the concentrate and the Working Wash Buffer in the refrigerator. Use within 14 days.

Storage/Stability

All components of this kit are stable at 2 to 8 $^{\circ}\text{C}$. Any unused reconstituted standard should be discarded or frozen at -70°C . Standard can be frozen and thawed one time only without loss of immunoreactivity.

Refer to the Certificate of Analysis for kit shelf life. To obtain C of A go to www.sigma-aldrich.com

Procedure

Precautions

- 20-30 minutes before use equilibrate kit and all reagents to room temperature (15-30 $^{\circ}\text{C}$).
- Use only the coated 96 well capture plate provided with the kit
- Multiwell plate: equilibrate to room temperature in unopened foil bag. Remove desired number of strips, reseal the bag and refrigerate at 2 - 8 $^{\circ}\text{C}$ to maintain plate integrity.
- When not in use all kit components should be refrigerated.
- Assay all standards, controls and samples in duplicate.
- If particulate matter is present, centrifuge or filter prior to analysis.
- A standard curve must be run with each assay
- Maintain a consistent order of components and reagents addition from well to well. This ensures equal incubation times for all wells.
- Run in-house controls with every assay. If control values fall outside pre-established ranges, the accuracy of the assay may be suspect.
- All reagents are lot-specific. Do not mix reagents from different kit lots.
- Do not use reagents past the kit shelf life.
- Standards and samples can be made up in either glass or plastic tubes.
- Pre-rinse the pipette tip with the reagent and use fresh pipette tips for each sample, standard or reagent.
- Read absorbances within 2 hours of assay completion.

Washing directions

- The purpose of washing is to remove unbound proteins and other non-specific parts of lysate.
- Incomplete washing will adversely affect the assay and render false results.
- Use only Wash Buffer provided in kit.
- Washing may be performed using automated washer, manifold pipette or squirt bottle.
- Wash cycle four times, blotting as dry as possible after the 4th wash.
- When washing manually, fill wells with Wash Buffer, aspirate thoroughly and tap dry on absorbent tissue.

Assay Procedure

- 1) **Incubate 100 μ L of Standards and Samples (diluted >1:10) for 2 hours at RT. (Optional: Incubate overnight at 4°C)**

 aspirate and wash 4x

- 2) **Incubate 100 μ L of Detection Antibody 1 hour at RT.**

 aspirate and wash 4x

- 3) **Incubate 100 μ L of Anti-Rabbit IgG-HRP 30 min at RT.**

 aspirate and wash 4x

- 4) **Incubate 100 μ L of stabilized Chromogen 30 minutes at RT (*in the dark*).**



- 5) **Add 100 μ L of Stop Solution and read at 450nm.**

Total Time 4 hours

p38 MAPK ELISA Assay Summary

Determine the number of wells for the assay run, including 2 zero wells, 2 chromogen blank wells, 14 standard dilutions wells and 2 wells for each sample to be assayed.

Remove appropriate number of multiwell strips and return the unused strips to the pouch. Reseal pouch.

1st incubation

- a. Add 100 μ L Standard Diluent to zero wells.
- b. Add 100 μ L standards, samples or controls to the appropriate wells. *Samples in Cell Extraction Buffer must be diluted at least 1:10 (1:50 or 1:100 may be necessary) in Standard Diluent Buffer. The dilutions should be optimized for each assay.*
- c. Tap gently on the plate to mix, cover with Plate Cover and incubate 2 hours at room temperature. *Alternatively, plate may be incubated overnight at 2 to 8 °C.*
- d. Wash wells 4 times following washing instructions.
- e. After the final wash blot dry on a lint free paper towel to remove any remaining wash buffer

2nd incubation

- a. Add 100 μ L Anti-p38 MAPK (detection) antibody to all wells (except chromogen blanks).
- b. Tap gently on the plate to mix, cover with Plate Cover and incubate 1 hour at room temperature.
- c. Wash wells for a total of 4 times following washing instructions.
- d. After the final wash blot dry on a lint free paper towel to remove any remaining wash buffer.

3rd incubation

- a. Add 100 μ L Anti-Rabbit IgG-HRP Working Solution to each well except the chromogen blanks.
- b. Cover with Plate Cover and incubate 30 minutes at room temperature.
- c. Wash wells for a total of 4 times following washing instructions.
- d. After the final wash blot dry on a lint free paper towel to remove any remaining wash buffer.

Substrate incubation

- a. Add 100 μ L of Stabilized Chromogen into all wells. *The liquid in the wells will begin to turn blue.*
- b. Do not cover the plate
- c. Incubate approximately 30 minutes at room temperature in the dark (*place plate in a drawer or cabinet*).

Note: *If your multiwell plate reader does not register optical density (OD) above 2.0, incubate only 20-25 minutes.*

Stop step

- a. Add 100 μ L of Stop Solution to each well. This stops the reaction
- b. Tap gently to mix. *The solution will turn yellow.*

Absorbance reading

- a. Any commercially available microplate reader capable of reading at OD 450 nm may be used.
- b. Blank the plate reader against the Chromogen Blank wells (contain Chromogen and Stop Solution).
- c. Read the absorbance of the entire plate at 450 nm within 2 hours after addition of Stop Solution.

Results

The results may be calculated using any immunoassay software package. The four-parameter algorithm provides the best curve fit. If the software program is not readily available, the concentrations of p38 MAPK may be calculated as follows:

1. Calculate the Average Net OD (nm) for each standard dilution and samples as follows:
Average Net OD (nm) =
Average Bound OD (nm) – Average Chromogen Blank OD (nm)
2. On graph paper plot the Average Net OD (nm) of standard dilution against the concentration (pg/mL) of p38 for the standards. Draw the best curve through these points to construct the standard curve.
3. The p38 concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by dilution factor of each sample.
5. Samples producing signals higher than the 2,000 pg/mL standard should be further diluted and assayed.

Product Profile

Typical Results

The standard curve below is for illustration only and **should not be used** to calculate results in your assay. Run standard curve in each assay.

OD 450 nm	p38 MAPK Standard (pg/mL)
0.248	0
0.310	31.2
0.363	62.5
0.451	125
0.613	250
0.887	500
1.470	1000
2.237	2000

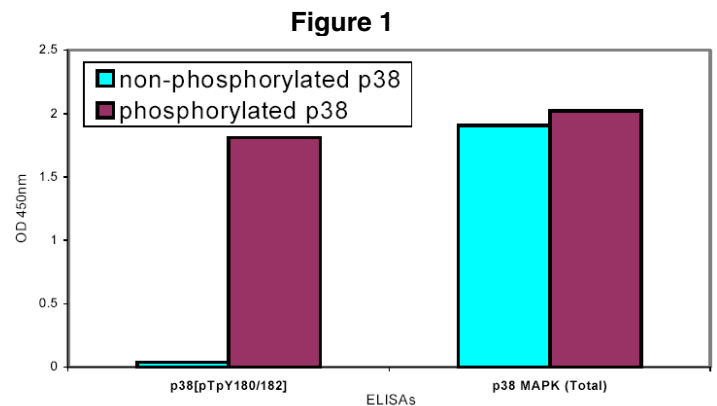
Limitations:

- Do not extrapolate the standard curve beyond the 2000 pg/mL standard point.
- The dose response is non-linear in this region and accuracy will be compromised.
- Other buffers and matrices have not been investigated.

Performance Characteristics

Specificity

The p38 MAPK ELISA is specific for measurement of human or mouse p38 MAPK protein independent of its phosphorylation state. As seen in Figure 1 p38 MAPK ELISA detected recombinant human p38 MAPK and p38 MAPK phosphorylated using MKK6 enzyme *in vitro*. At the same time, Phospho-p38 MAPK (pThr¹⁸⁰/pTyr¹⁸²) ELISA (Product No.CS0020) detected p38 MAPK phosphorylated on threonine 180 and tyrosine 182, but did not detect non-phosphorylated p38.



The p38 MAPK ELISA also detects both phosphorylated and non-phosphorylated p38 MAPK in Jurkat cells, whereas the Phospho-p38 MAPK (pThr¹⁸⁰/pTyr¹⁸²) ELISA detects only phosphorylated p38 MAPK in anisomycin treated Jurkat cells. ELISA and immunoblot results yielded equivalent data (Figure 2)

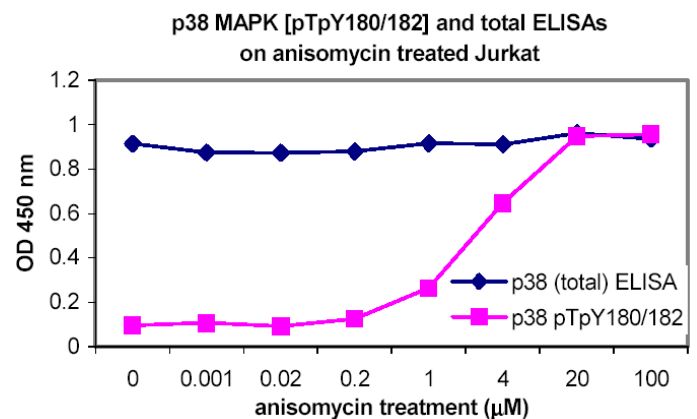


Figure 2

Sensitivity

Sensitivity of this assay is <16 pg/mL of human p38 MAPK. Sensitivity was calculated by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times. In Jurkat cells cultured in complete medium, this sensitivity corresponded to the p38 MAPK protein extractable from 1000 cells/well.

The sensitivity of this ELISA was compared to immunoblotting using known quantities of p38 MAPK.

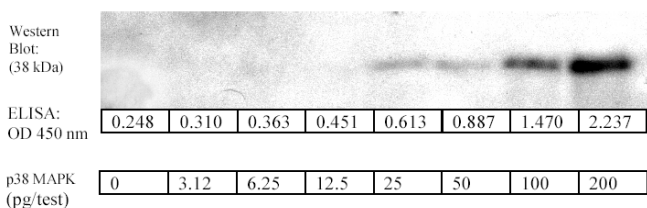


Figure 3

Detection of p38 MAPK by ELISA vs. immunoblot

The results show that ELISA is approximately 10 times more sensitive in detecting p38 than immunoblotting.

Precision

1. Intra-Assay Precision

Samples of known p38 MAPK concentration were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	881	242	67
Standard Deviation (SD)	37	9	5
% Coefficient of Variation	4.2	3.9	7.3

2. Inter-Assay Precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	934	232	70
Standard Deviation (SD)	63	14	7
% Coefficient of Variation	6.7	5.8	9.5

Sample Recovery

To evaluate recovery, rat brain tissue was extracted with cell lysate buffer and the extract adjusted to 200 µg/mL total protein. Recombinant p38 MAPK was spiked into the extract at 3 levels and the percent recovery over endogenous levels calculated. On average, 93% recovery was observed.

Parallelism

Natural p38 MAPK from human colo205 and 293 cell lysates were serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the p38 MAPK standard curve. Parallelism was demonstrated by the figure below that indicated that the standard accurately reflects p38.

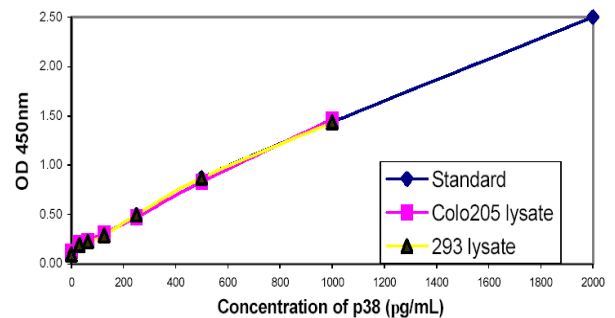


Figure 4 p38 ELISA: Parallelism

Linearity of Dilution

Jurkat cells were grown in tissue culture medium containing 10% fetal calf serum and lysed with Cell Extraction Buffer. This lysate was diluted in Standard Diluent Buffer over the range of the assay and measured for p38 MAPK content. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

Cell Lysate			
Dilution	Measured (pg/mL)	Expected (pg/mL)	% Expected
Neat	966	966	100
1:2	494	483	102
1:4	232	246	94
1:8	120	116	103
1:16	66	60	109

References

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