ELISA Kit
Catalog #KHO0061

# p38 MAPK (Total)

www.invitrogen.com
Invitrogen Corporation
542 Flynn Road, Camarillo, CA 93012
Tel: 800-955-6288
E-mail: techsupport@invitrogen.com

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## INTRODUCTION

p38 MAP kinase (MAPK), also known as RK (CDC2-related protein kinase) or CSBP (cytokine suppressive anti-inflammatory drug binding protein), is the mammalian homologue of the yeast HOG kinase (high osmolarity glycerol response kinase). There are at least three distinct mitogen-activated protein kinase (MAP kinase) signaling modules which mediate extracellular signals into the nucleus to turn on the responsive genes in mammalian cells, including extracellular mitogen-regulated kinase (ERK), c-Jun $NH_2$-terminal kinase (JNK, also called stress-activated protein kinase, SAPK), and p38 kinase. The p38 signaling transduction pathway plays an essential role in regulating many cellular processes including inflammation, cell differentiation, cell growth, and death.

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. Recently, MAPKK-independent activation of p38 was shown to involve the interaction of p38 with TAB1 [transforming growth factor-beta-activated protein kinase 1 (TAK1)-binding protein 1] leading to autophosphorylation and activation of p38. Various targets of p38 have been identified including transcription factors ATF-2, Max, MEF2C, CHOP, MAPKAPK2 (MAPK-activated protein kinase-2), and PRAK kinase (p38-related/ activated protein kinase).

p38 MAPK is expressed broadly in normal tissues and various cell lines. There are three alternatively spliced forms of p38 (CSBP2/p38α, CSBP1, and Mxi2) as well as several homologues including p38β, p38β 2, p38γ, and p38δ. These homologues are expressed at different levels in human tissues and can be activated by different, although sometimes overlapping, stress stimuli.

The Invitrogen p38 MAPK (Total) ELISA is designed to detect and quantify the levels of p38 MAPK protein independent of its phosphorylation state. Cross-reaction of human, monkey and mouse p38 MAPK protein in this assay is documented. The reactivity of this ELISA with other species has not been tested. This assay is intended to detect p38 MAPK from lysates of cells and can be used to normalize the p38 MAPK content of the samples when examining quantities of phosphorylated sites on p38 MAPK using other Invitrogen kits (Cat. # KHO0071).

**For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.**

**READ ENTIRE PROTOCOL BEFORE USE**

## PRINCIPLE OF THE METHOD

The Invitrogen p38 MAPK (Total) kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for p38 MAPK (regardless of phosphorylation state) has been coated onto the wells of the microtiter strips provided. Samples, including a standard containing p38 MAPK, control specimens, and unknowns, are pipetted into these wells. During the first incubation, the p38 MAPK antigen binds to the immobilized (capture) antibody. After washing, 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 all the 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.

## REAGENTS PROVIDED

**Note:** *Store all reagents at 2 to 8°C.*

| ***Reagent*** | ***96 Test Kit*** |
|---|---|
| *p38 MAPK (Total) Standard*: Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume. | 2 vials |
| *Standard Diluent Buffer*. Contains 0.1% sodium azide; 25 mL per bottle. | 1 bottle |
| *Antibody Coated Well*s, 12x8 Well Strips. | 1 plate |
| *p38 MAPK (Total) Detection Antibody*. Contains 0.1% sodium azide; 11 mL per bottle. | 1 bottle |
| *Anti-Rabbit IgG HRP (100X).* Contains 3.3 mM thymol; 0.125 mL per vial. | 1 vial |
| *HRP Diluent.* Contains 3.3 mM thymol; 25 mL per bottle. | 1 bottle |
| *Wash Buffer Concentrate (25X)*; 100 mL per bottle. | 1 bottle |
| *Stabilized Chromoge*n, *Tetramethylbenzidine (TMB)*; 25 mL per bottle. | 1 bottle |
| *Stop Solutio*n; 25 mL per bottle. | 1 bottle |
| *Plate Covers*, adhesive strips. | 3 |

**Disposal Note:** This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

## SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Cell Extraction Buffer (see Recommended Formulation, p.11).
4. Distilled or deionized water.
5. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
6. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
7. Glass or plastic tubes for diluting and aliquoting standard.
8. Absorbent paper towels.
9. Calibrated beakers and graduated cylinders in various sizes.

## PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bag.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.

3. 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.
4. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
5. It is recommended that all standards, controls and samples be run in duplicate.
6. 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.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
8. Cover or cap all reagents when not in use.
9. **Do not mix or interchange different reagent lots from various kit lots**.
10. Do not use reagents after the kit expiration date.
11. Read absorbances within 2 hours of assay completion.
12. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
13. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
14. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

## SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

## DIRECTIONS FOR WASHING

**Incomplete washing will adversely affect the test outcome.** All washing must be performed with *Wash Buffer Concentrate (25X)* provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed. If your automated washer allows, 30 second soak cycles should be programmed into the wash cycle.

## PROCEDURE FOR EXTRACTION OF PROTEINS FROM CELLS

### A. Recommended Formulation of Cell Extraction Buffer:

10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM $Na_3VO_4$
1% Triton X-100
10% glycerol
0.1% SDS
0.5% deoxycholate
1 mM PMSF (stock is 0.3 M in DMSO)
Protease inhibitor cocktail (ex., Sigma Cat. # P-2714) (reconstituted according to manufacturer's guideline). Add 250 µL per 5 mL Cell Extraction Buffer.

This buffer is stable for 2 to 3 weeks at 4°C or for up to 6 months when aliquoted (without protease inhibitors and PMSF added) and stored at -20°C. When stored frozen, the Cell Extraction Buffer should be thawed on ice. Important: add the protease inhibitors just before using. The stability of protease inhibitor supplemented Cell Extraction Buffer is 24 hours at 4°C. PMSF is very unstable and must be added prior to use, even if added previously.

## B. Protocol for Cell Extraction

This protocol has been applied to several cell lines with the Cell Extraction Buffer above. Researchers may optimize the cell extraction procedures that work best in their hands.

1. Collect cells in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).
2. Wash cells 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 -80$^{o}$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, $10^{8}$ Jurkat cells grown in RPMI plus 10% FBS can be extracted in 1 mL of Extraction Buffer. Under these conditions, use of 0.1-1 µL of the clarified cell extract diluted to a volume of 100 µL/well in Standard Diluent Buffer (See **Assay Method**) is sufficient for the detection of p38 MAPK.
5. Transfer extracts to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4$^{o}$C.
6. Aliquot the clear lysate to clean microfuge tubes. These samples are ready for assay. Lysates can be stored at -80$^{o}$C. Avoid multiple freeze/thaw cycles.

## REAGENT PREPARATION AND STORAGE

### A. Reconstitution and Dilution of p38 MAPK (Total) Standard

**Note:** This *p38 MAPK (Total)* standard was prepared from purified full length recombinant human p38 MAPK protein expressed in *E. coli*.

1. Reconstitute *p38 MAPK (Total) Standard* with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Label as 2000 pg/mL p38 MAPK. Use standard within 1 hour of reconstitution.
2. Add 0.25 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 1000, 500, 250, 125, 62.5, and 31.2 pg/mL p38 MAPK.
3. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

**B. Dilution of p38 MAPK (Total) Standard**

| Standard: | Add: | Into: |
|---|---|---|
| 2000 pg/mL | Prepare as described in step 1 | |
| 1000 pg/mL | 0.25 mL of the 2000 pg/mL std. | 0.25 mL of the Diluent Buffer |
| 500 pg/mL | 0.25 mL of the 1000 pg/mL std. | 0.25 mL of the Diluent Buffer |
| 250 pg/mL | 0.25 mL of the 500 pg/mL std. | 0.25 mL of the Diluent Buffer |
| 125 pg/mL | 0.25 mL of the 250 pg/mL std. | 0.25 mL of the Diluent Buffer |
| 62.5 pg/mL | 0.25 mL of the 125 pg/mL std. | 0.25 mL of the Diluent Buffer |
| 31.2 pg/mL | 0.25 mL of the 62.5 pg/mL std. | 0.25 mL of the Diluent Buffer |
| 0 pg/mL | 0.25 mL of the Diluent Buffer | An empty tube |

Remaining reconstituted standard should be discarded or frozen at -80$^{o}$C. for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.

### C. Storage and Final Dilution of Anti-Rabbit IgG HRP (100X)

**Please Note:** The *Anti-Rabbit IgG HRP (100X)* is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow *Anti-Rabbit IgG HRP (100X)* to reach room temperature. Gently mix. Pipette *Anti-Rabbit IgG HRP (100X)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µL of this 100X concentrated solution with 1 mL of *HRP Diluent* for each 8-well strip used in the assay. Label as Anti-rabbit IgG HRP Working Solution.

For Example:

| # of 8-Well Strips | Volume of Anti-rabbit IgG HRP (100X) | Volume of Diluent |
|---|---|---|
| 2 | 20 µL solution | 2 mL |
| 4 | 40 µL solution | 4 mL |
| 6 | 60 µL solution | 6 mL |
| 8 | 80 µL solution | 8 mL |
| 10 | 100 µL solution | 10 mL |
| 12 | 120 µL solution | 12 mL |

2. Return the unused *Anti-Rabbit IgG HRP (100X)* to the refrigerator.

### D. Dilution of Wash Buffer

Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

## ASSAY METHOD: PROCEDURE AND CALCULATIONS

**Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.**

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

**Note:** A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 100 µL of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 100 µL of standards, samples or controls to the appropriate microtiter wells. Samples prepared in Cell Extraction Buffer must be diluted 1:10 or greater in *Standard Diluent Buffer* (for example, 10 µL sample into 90 µL buffer). While a 1:10 sample dilution has been found to be satisfactory, higher dilutions such as 1:50 or 1:100 may be optimal. The dilution chosen should be optimal for

each experimental system. Tap gently on side of plate to thoroughly mix. (See **REAGENT PREPARATION AND STORAG**E, Section B.)

4. Cover plate with *plate cover* and incubate for **2 hours at room temperature**. Alternatively, the plate may be incubated overnight at 4°C.
5. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
6. Pipette 100 µL of *p38 MAPK (Total*) *Detection Antibod*y solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
7. Cover plate with *plate cover* and incubate for **1 hour at room temperature.**
8. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
9. Add 100 µL *Anti-Rabbit IgG HRP* Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE**, Section C.)
10. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
11. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
12. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.

13. Incubate for **30 minutes at room temperature and in the dark**. ***Please Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
14. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
15. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution.*
16. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from *all* data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
17. Read the p38 MAPK (Total) concentrations for unknown samples and controls from the standard curve plotted in step 16. **Multiply value(s) obtained for sample(s) by dilution factor to correct for the dilution in step 3.** (Samples still producing signals higher than

the highest standard (2000 pg/mL) should be further diluted in *Standard Diluent Buffer* and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

**TYPICAL DATA**

The following data were obtained for the various standards over the range of 0 to 2000 pg/mL p38 MAPK.

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

## LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 2000 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >2000 pg/mL with *Standard Diluent Buffer*; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various extraction buffers has not been thoroughly investigated. The rate of degradation of native p38 MAPK in various matrices has not been investigated.

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## PERFORMANCE CHARACTERISTICS

### SENSITIVITY

The analytical sensitivity of this assay is <16 pg/mL of human Total p38 MAPK. This was determined 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 Western blotting using known quantities of p38 MAPK. The data presented in Figure 1 show that the sensitivity of the ELISA is approximately 10x greater than that of Western blotting. The bands shown in the Western blot data were developed using rabbit anti-p38 MAPK, an alkaline phosphatase conjugated anti-rabbit IgG followed by chemiluminescent substrate and autoradiography.

**Figure 1: Detection of p38 MAPK by ELISA vs Western Blot:**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ELISA: OD 450 nm | 0.248 | 0.310 | 0.363 | 0.451 | 0.613 | 0.887 | 1.470 | 2.237 |
| p38 MAPK (pg/test) | 0 | 3.12 | 6.25 | 12.5 | 25 | 50 | 100 | 200 |

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 |
| SD | 37 | 9 | 5 |
| %CV | 4.2 | 3.9 | 7.3 |

SD = Standard Deviation
CV = Coefficient of Variation

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 |
| SD | 63 | 14 | 7 |
| %CV | 6.7 | 5.8 | 9.5 |

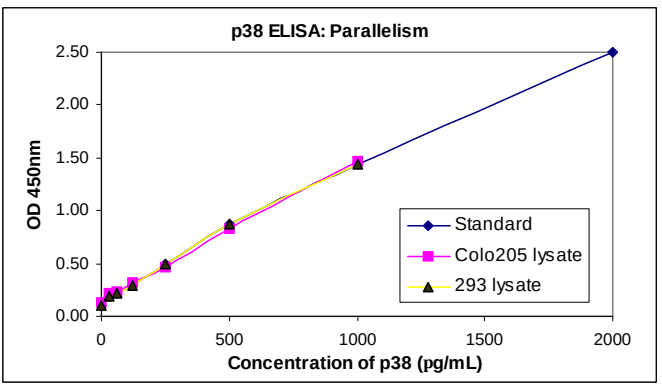
SD = Standard Deviation
CV = Coefficient of Variation

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 and indicated that the standard accurately reflects p38 MAPK content in samples.



LINEARITY OF DILUTION

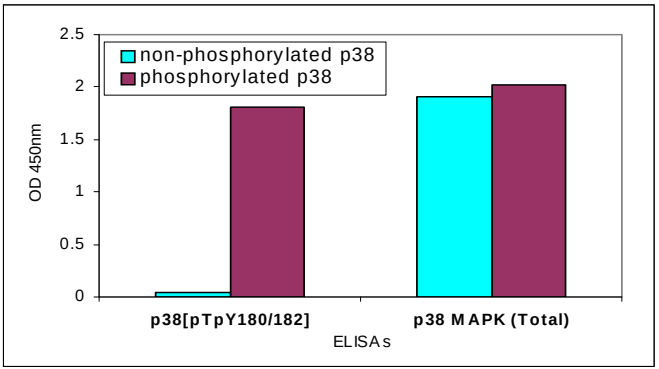
Jurkat cells were grown in tissue culture medium containing 10% fetal bovine serum and lysed with Cell Extraction Buffer. This lysate was diluted in *Standard Diluent Buffer* over the range of the assay and measured for p38 MAPK content. Linear regression analysis of samples versus the expected concentration 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 |

SPECIFICITY

The p38 MAPK (Total) ELISA kit is specific for measurement of human or mouse p38 MAPK protein. Recombinant human 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 p38 MAPK [pTpY180/182] ELISA (Cat. # KHO0071) and p38 MAPK (Total) ELISA.

**Figure 2**

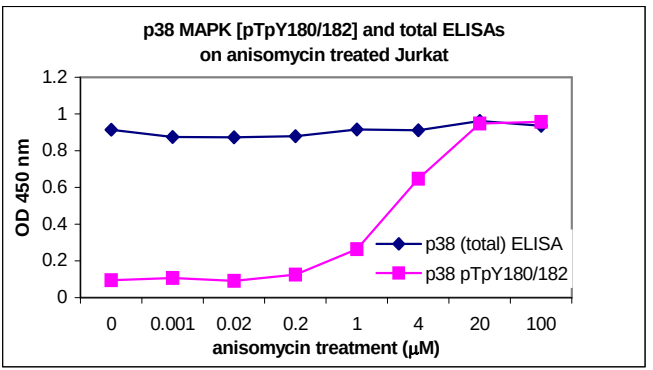


This "Total" assay is designed to allow normalization of p38 MAPK content among samples to permit interpretation of results from Phosphorylation Site-Specific p38 MAPK kits available from Invitrogen Corporation.

Figure 3 shows the results obtained from a dose response of anisomycin treatment on Jurkat cells. The data indicate that the p38 MAPK (Total) ELISA detects both phosphorylated and non-phosphorylated p38 MAPK in Jurkat cells, whereas the p38 MAPK [pTpY180/182] ELISA detects phosphorylated p38 MAPK in anisomycin treated cells. ELISA and Western blot results yield equivalent data.

**Figure 3**


Western Blot
Total p38 MAPK
p38 MAPK
[pTpY180/182]


p38 MAPK [pTpY180/182] and total ELISAs
on anisomycin treated Jurkat
OD 450 nm
1.2
1
0.8
0.6
0.4
0.2
0
0
0.001
0.02
0.2
1
4
20
100
anisomycin treatment (μM)
p38 (total) ELISA
p38 pTpY180/182

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**Explanation of symbols**

| Symbol | Description | Symbol | Description |
|---|---|---|---|
|  | Catalogue Number |  | Batch code |
|  | Research Use Only |  | *In vitro* diagnostic medical device |
|  | Use by |  | Temperature limitation |
|  | Manufacturer |  | European Community authorised representative |
| [-] | Without, does not contain | [+] | With, contains |
|  | Protect from light |  | Consult accompanying documents |
|  | Directs the user to consult instructions for use (IFU), accompanying the product. | | |

**NOTES**

# p38 MAPK (Total) Assay Summary

Incubate 100 µL Standard or Cell Extract (>1:10) for 2 hours at RT

**aspirate and wash 4x**

Incubate 100 µL of Detection Antibody for 1 hour at RT

**aspirate and wash 4x**

Incubate 100 µL of HRP Anti-Rabbit Antibody for 30 minutes at RT

**aspirate and wash 4x**

Incubate 100 µL of Stabilized Chromogen for 30 minutes at RT

Add 100 µL of Stop Solution and read at 450 nm

**Total time: 4 hours**