

## Product Information

### Phospho-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] ELISA

Catalog Number **CS0650**

Storage Temperature 2-8 °C

### Technical Bulletin

#### Product Description

Phospho-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] ELISA is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) for quantitative determination of MEK1 protein in cell lysates. A monoclonal antibody specific for MEK1 (regardless of phosphorylation state) has been coated onto the wells of the multiwell plate strips provided. MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] standard dilutions, control specimens, and unknown samples are pipetted into these wells. During the first incubation, the phospho-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] antigen binds to the immobilized (capture) antibody. After incubation, the excess reagents are washed away and an antibody specific for MEK1 phosphorylated on serine residues 218 and 222 is added to the wells. During the second incubation, this antibody serves as a detection antibody by binding to the immobilized MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>]. After removal of excess detection antibody, horseradish peroxidase-labeled (HRP) anti-rabbit IgG 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 MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] present in the original specimen. The optical density measured at 450 nm in the multiwell plate reader is used to calculate the concentration of phospho-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>].

The Phospho-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] ELISA is designed to detect and quantify the level of MEK1 protein when dually phosphorylated at serine residues 218 and 222. This assay is intended for the detection of MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] from lysates of human and mouse cells. For normalizing the MEK1 content of the samples, a MEK1 ELISA, which permits quantification of MEK1 independent of phosphorylation status, is available from Sigma (Catalog No. CS0700).

MEK1, also known as ERK kinase 1, MAPK kinase 1, and MKK1, is a member of the MEK family of dual specificity protein kinases, with MW = 45 kDa. MEK1 shares approximately 80% homology with the closely

related dual specificity protein kinase MEK2. MEK family members are homologous with the *byr1* gene product of *Schizosaccharomyces pombe* and share the common feature of phosphorylating the threonine and tyrosine residues of a conserved (T-X-Y) motif within the activation loop of substrate proteins. For MEK1, the phosphorylated threonine and tyrosine residues are located within the sequence Thr-Glu-Tyr (TEY). MEK1 contains an N-terminal ERK docking domain (D domain), an N-terminal regulatory region, a consensus catalytic core, and a nuclear export sequence (NES). Under resting conditions, MEK1 localizes to the cytoplasm, but translocates to the nucleus in response to mitogenic stimuli, or to membrane-proximal adhesion structures in response to adhesion.

MEK1 is a key component of the ERK1/2 signaling cascade. The series of events leading to ERK1/2 phosphorylation in this cascade has been an active area of research for over 10 years. In response to growth factor, cytokine, or hormone binding with its specific receptor, membrane-associated Ras is loaded with GTP and thus activated. Activated Ras then recruits members of the Raf protein family (Raf-1, A-Raf, and B-Raf) to the plasma membrane. The interaction of Raf-1 with Ras at the plasma membrane results in phosphorylation of multiple sites on Raf-1 (tyrosines 340 and 341) and interaction with several other proteins, including 14-3-3, Hsp90 and Hsp50. These phosphorylation events and protein-protein interactions relieve the autoinhibitory effect of the Raf N-terminal domain and increase the catalytic activity and membrane localization of Raf. Active Raf family members phosphorylate MEK1 at serines 218 and 222, thereby activating it.

Activated MEK1 (along with MEK2) in turn phosphorylates ERK1 and ERK2 at the conserved threonine and tyrosine residues of the activation loop. ERK1 and ERK2 are the only known substrates for MEK1. The mechanism by which the dual phosphorylation occurs is nonprocessive, i.e., ERK is observed to associate with MEK1 and become phosphorylated at the first residue, then must

dissociate from MEK1, and re-associate with MEK1 in order for phosphorylation of the second site to occur, as revealed by kinetic studies. Upon phosphorylation, ERK then translocates from the cytoplasm to the nucleus, possibly following dimerization. In the nucleus, ERK phosphorylates and activates Elk-1, Sap-1a, Ets1, c-Myc, Tal, and signal transducer and activator of transcription (STAT) proteins. Phosphorylation of Elk-1 by ERK enhances Elk-1's affinity for the serum response element and enhances transcription of growth related proteins, such as c-Fos. In addition to transcription factors, ERK also phosphorylates p90rsk, which in turn activates another series of transcription factors, as well as other proteins including cytosolic phospholipase A2, the juxtamembrane region of the EGF receptor, and several microtubule-associated proteins (MAP), including MAP-1, MAP-2, MAP-4, and Tau. MEK1 is also phosphorylated by the kinases c-mos, MEK kinase-1, cdk5/p35, and PAK1 (p21-activated protein kinase). PAK1 phosphorylates MEK1 at serine 298 and possibly threonine 292 by a mechanism that does not require membrane recruitment, providing a site of convergence between integrin and growth factor signaling. This may explain the need for cell adhesion in growth factor signaling.

MEK's involvement in regulating the ERK1/2 MAPK signaling cascade is currently under investigation in many areas of research including control of cell proliferation, cell cycle and mitosis, organelle distribution during mitosis, immune responses, protection from apoptosis in response to Fas, TRAIL, ischemia and other stressors, cell differentiation, and in the control of transcription. The signaling cascade is also under intensive investigation in cancer studies, tumorigenesis, and oncogenic transformation. Activation of ERK1 and 2 is correlated with invasiveness and metastatic potential. PD98059, a MEK1-specific antagonist, used alone and in conjunction with chemotherapeutic agents is currently under investigation in many cancer studies.

#### Reagents

- **Phospho-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] Standard, Lyophilized, 2 vials, Catalog No. P3997** – purified full length recombinant phosphorylated MEK1. Refer to vial label for quantity and reconstitution volume.
- **Standard Diluent Buffer, 25 mL, Catalog No. S3068**, contains sodium azide as preservative.
- **Sample Treatment Buffer, 10 mL, Catalog No. S9694**
- **Monoclonal-Anti-MEK1 Coated 96 well plate, 1EA, Catalog No. M8068** - A plate using break-apart strips coated with monoclonal antibody specific for full-length MEK1 (regardless of phosphorylation state).
- **Anti-phospho-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>], 5.5 mL, Catalog No. P3872**  
A detection antibody, produced in rabbit. Contains sodium azide. Ready to use.
- **Anti-Rabbit IgG-HRP, Concentrate (100X), 1 vial, Catalog No. R7653** - contains 3.3 mM thymol and 50% glycerol, viscous. *See Reagent Preparation for handling, dilution and storage instructions.*
- **HRP Diluent, 25 mL, Catalog No. H5788** - contains 3.3 mM thymol. Ready to use.
- **Wash Buffer concentrate, 25X, 100 mL, Catalog No. W2639** - *See Reagent Preparation for handling, dilution and storage instructions*
- **Stabilized Chromogen, Tetramethylbenzidine (TMB), 25 mL, Catalog No. S3318** –Avoid prolonged exposure to light. Avoid exposure to metal. Ready to use.
- **Stop Solution, 25 mL, Catalog No. S2818** – Ready to use.
- **Plate Covers, Adhesive strips, 3 each, Catalog No. P4870**

#### 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.
- 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.
- Graph paper: linear, log-log, or semi-log, as desired.

### 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 Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>  
 2 mM Na<sub>3</sub>VO<sub>4</sub>  
 1% Triton<sup>®</sup> X-100  
 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, Catalog No. P2714  
 Add 250 µl of reconstituted cocktail per 5 mL of Cell Extraction Buffer.

Stable for 2-3 weeks at 2-8 °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

This protocol has been successfully applied to several cell lines of human origin. Researchers may use the procedures that work best in their hands. They will have to assay their 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.
5. The volume of Cell Extraction Buffer depends on the cell number in cell pellet and expression of MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>]. For example, 4X10<sup>6</sup> Jurkat cells grown in DMEM plus 10% FBS and treated with 100 ng/mL PMA for 10 minutes can be extracted in 1 mL of Cell Extraction Buffer. Under these conditions, use of 1-10 µL of the clarified cell extract diluted to a volume of 50 µL/well in Standard Diluent Buffer is sufficient for the detection of MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>].
6. Transfer extracts to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 2-8 °C.
7. Aliquot the clear lysate to clean microcentrifuge tubes

### Sample Pre-Treatment:

Incubate each sample and control with an equal volume of *Sample Treatment Buffer* on ice for 20 minutes. Dilute this mixture at least 5-fold in *Standard Diluent Buffer*. For example, for duplicate analyses, add 10 µL sample and 10 µL *Sample Treatment Buffer*, then after incubation, add 80 µL *Standard Diluent Buffer*. The dilution chosen should be optimized for each experimental system.

### Reagent Preparation

#### Standard

*Note:* This Phospho-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] standard was prepared using purified, full-length, recombinant MEK1 protein

**One Unit** of standard is equivalent to the amount of MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] derived from 250 pg of total MEK1 protein.

1. Reconstitute MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] Standard with *Standard Diluent Buffer*. Refer to standard vial label for instructions.
2. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution.
3. Label as 100 units/mL MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>]. Use standard within 1 hour of reconstitution.

4. Prepare serial standard dilutions as follows:

Tube #	Standard Buffer	Standard from tube #:	Final MEK1 ng/mL
1	Reconstitute according to label instructions		100 units /mL
2	0.15 mL	0.15 mL (1)	50 units/mL
3	0.15 mL	0.15 mL (2)	25 units/mL
4	0.15 mL	0.15 mL (3)	12.5 units/mL
5	0.15 mL	0.15 mL (4)	6.25 units/mL
6	0.15 mL	0.15 mL (5)	3.12 units/mL
7	0.15 mL	0.15 mL (6)	1.6 units /mL
8	0.15 mL	-	0 units /mL

5. Remaining reconstituted standard should be discarded or frozen at  $-70^{\circ}\text{C}$ . for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.

#### Anti-rabbit IgG Horseradish Peroxidase (HRP)

*Note:* The *Anti-rabbit IgG-HRP* 100X concentrate is in 50% glycerol. This solution is viscous. To ensure accurate dilution:

1. Equilibrate to room temperature, mix gently, pipette slowly.
2. Remove excess concentrate solution from pipette tip with clean absorbent paper.
3. Within 1 hour of use, dilute 10  $\mu\text{L}$  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.
4. Return the unused concentrate to the refrigerator
5. For more strips use the following amounts:

# of 8 well strips	IgG-HRP Concentrate mL	Diluent mL
2	20	2
4	40	4
6	60	6
8	80	8
10	100	10
12	120	12

#### 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  $2-8^{\circ}\text{C}$ . Any unused reconstituted standard should be discarded or frozen at  $-70^{\circ}\text{C}$ . 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](http://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 component and reagent 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 after the kit expiration date.
- 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 4<sup>th</sup> wash.
- When washing manually, fill wells with Wash Buffer, aspirate thoroughly and tap dry on absorbent tissue.
- It is recommended to use laboratory tape to hold plate strips to the plate frame while performing the plate washing and drying procedure to avoid strips coming free of the frame.

### Assay Procedure

#### Phospho-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] ELISA Assay Summary

- 1) **Add 50 mL of MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] Standards or Samples (samples diluted 1:10 or higher in Standard Diluent Buffer) to appropriate wells**  
**Add 50 mL Anti-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>]**

**Incubate 2 hours at RT**  
**aspirate and wash 4x**

- 2) **Add 100 mL Anti-Rabbit IgG-HRP**

**Incubate 30 minutes at RT.**  
**aspirate and wash 4x**

- 3) **Add 100 mL Stabilized Chromogen**

**Incubate 30 minutes at RT**  
**(in the dark).**

- 4) **Add 100 mL of Stop Solution**  
**Read at 450nm.**

**Total Assay Time - 3 hours**

- Determine the number of wells for the assay run, including 2 zero wells, 2 chromogen blank wells, 14 standard dilution 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

#### 1<sup>st</sup> incubation

- a Add 50  $\mu$ L Standard Diluent to zero wells.
- b Add 50  $\mu$ L MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] standards, samples or controls to the appropriate wells.
- c Samples prepared in cell extraction buffer or brain homogenate buffer must be diluted 1:10 or greater in *Standard Diluent Buffer* (for example, 10  $\mu$ L sample plus 90  $\mu$ L buffer). *The dilutions should be optimized for each assay.*
- d Cell culture supernatants or buffered solutions; dilute 1:2 in *Standard Diluent Buffer* (50  $\mu$ L buffer + 50  $\mu$ L sample).
- e Add 50  $\mu$ L Anti-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] detection antibody to all wells (except chromogen blanks).
- f Tap gently on the plate to mix, cover with Plate Cover and incubate 2 hours at room temperature.
- g Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells for a total of 4 times following washing instructions

#### 2<sup>nd</sup> incubation

- a Add 100  $\mu$ 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  $\mu$ 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 reaction

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

### Absorbance reading

- Any commercially available multiwell plate reader capable of reading at OD 450 nm may be used.
- Blank the plate reader against the Chromogen Blank wells (containing Chromogen and Stop Solution).
- 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 MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] may be calculated manually.
- Calculate the Average Net OD (average reading of 2 wells) for each standard dilution and samples as follows:
- Average Net OD = Average Bound OD – Average Chromogen Blank OD
- On graph paper plot the Average Net OD of standard dilutions against the standard concentration (units/mL) of MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>]. Draw the best curve through these points to construct the standard curve.
- The MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
- Multiply the values obtained for the samples by dilution factor of each sample.
- Samples producing signals higher than the 100 units/mL standard should be further diluted and assayed again.

### 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.

MEK1 [pSer <sup>218</sup> /pSer <sup>222</sup> ] units/mL	OD 450 nm
100	2.76
50	1.32
25	0.72
12.5	0.45
6.25	0.30
3.12	0.23
1.6	0.19
0	0.13

### Limitations

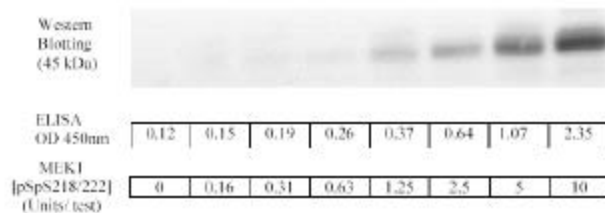
- Do not extrapolate the standard curve beyond the 100 units/mL standard point.
- The dose response is non-linear in this region and accuracy will be compromised.
- Other buffers and matrices have not been investigated.
- The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of cell extract and brain homogenate samples have not been thoroughly investigated.
- The rate of degradation of native MEK1 or dephosphorylation of MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] in various matrices has not been investigated.
- Although degradation of native MEK1 or dephosphorylation of MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] in the Cell Extraction Buffer described in this protocol has not been seen to date, the possibility of this occurrence cannot be excluded.

### Performance characteristics

#### Sensitivity

The analytical sensitivity of this assay is <0.9 unit/mL of MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>]. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times. Using Jurkat cells, this level of sensitivity was equivalent to the detection of MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] in 4000 cells.

The sensitivity of this ELISA was compared to immunoblotting using known quantities of MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>]. The data presented in Figure 1 show that Phospho-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] ELISA is approximately four times as sensitive as immunoblotting. The bands shown in the immunoblotting were developed using rabbit anti-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>], an alkaline phosphatase conjugated anti-rabbit IgG followed by chemiluminescent substrate.



**Figure 1 Detection of MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] by ELISA vs immunoblot**

### Precision

#### 1. Intra-Assay Precision

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

	Sample 1	Sample 2	Sample 3
Mean (units/mL)	72.4	42.9	15.7
Standard Deviation (SD)	3.8	2.4	0.5
% Coefficient of Variation	5.3	5.7	3.3

#### 2. Inter-Assay Precision

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

	Sample 1	Sample 2	Sample 3
Mean (units/mL)	73.5	44.0	16.9
Standard Deviation (SD)	6.2	3.8	1.5
Coefficient of Variation %	8.4	8.7	9.0

### Recovery

To evaluate recovery, phospho-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] Standard was spiked at 3 different concentrations into 10% cell extraction buffer. The average recovery was 117%.

### Parallelism

Natural MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] from PMA-treated Jurkat cell lysate was serially diluted in *Standard Diluent Buffer*. The optical density of each dilution was plotted against the MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] standard curve. Parallelism demonstrated by the figure below indicated that the standard accurately reflects MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] content in samples.

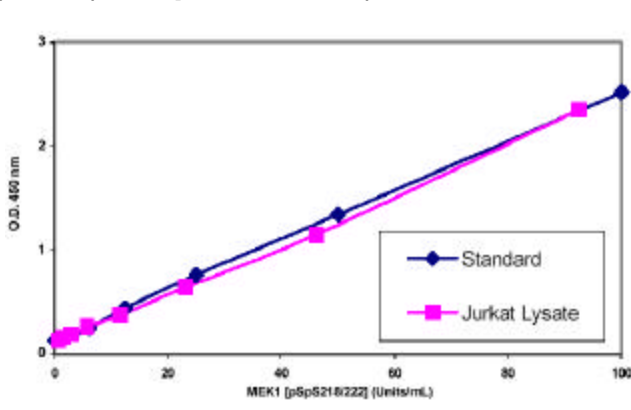


Figure 2 Parallelism: MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>]

### Linearity of Dilution

Jurkat cells were grown in cell culture medium containing 10% FCS, treated with 100 ng/mL PMA for 10 minutes and lysed with Cell Extraction Buffer. This lysate was pretreated and then diluted with *Standard Diluent Buffer* over the range of the assay and measured for MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] content. The neat dilution corresponds to a 1:10 final, in well dilution of cell lysate. Linear regression analysis of sample values versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Cell Lysate		
	Measured units/mL	Expected units/mL	% Expected
Neat	92.5	92.5	100
1:2	39.2	46.3	84.8
1:4	21.5	23.1	93.0
1:8	10.1	11.6	87.4
1:16	5.6	5.8	96.9
1:32	2.5	2.9	86.5
1:64	1.5	1.4	103.8

### Specificity

The Phospho-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] ELISA recognizes both human and mouse MEK1. Other species have not been tested.

The specificity of this assay for dually phosphorylated MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] was confirmed by peptide competition. The data presented in Figure 3 show that only the dual phospho-peptide containing the phosphorylated serines could block the ELISA signal. The non-phosphorylated peptide sequence or mono-phosphopeptide [pSer<sup>222</sup>] did not block the signal by more than 10%.

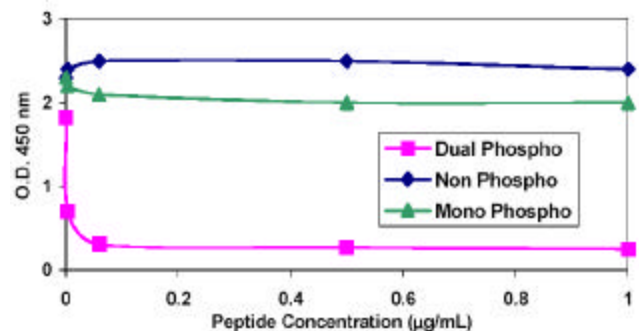
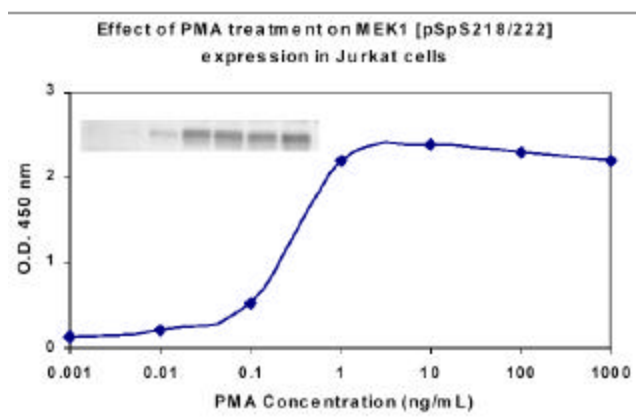


Figure 3 Peptide blocking: Phospho-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>]

- Jurkat cells were treated with PMA at varying concentrations (0.001 to 100 ng/mL) for 10 minutes.
- Then the cells were lysed and quantitated for MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>].
- The level of MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] increases with dosage of PMA.
- The results correlated very well with immunoblot analysis of the same samples (see insert in the graph below).



**Figure 4 PMA treatment and Phospho-MEK1 [pSer<sup>218</sup>/pSer<sup>222</sup>] expression**

4. Kolch, W., et al., Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions., *Biochem. J.* **351**, 289-305 (2000).
5. Bondzi, C., et al. A novel assay of the measurement of the Raf-1 kinases activity. *Oncogene*, **19**, 5030-5033 (2000).
6. Colanzi, A., et al., RAF-1-activated MEK1 is found on the Golgi apparatus in late prophase and is required for Golgi complex fragmentation in mitosis. *J. Cell Biol.*, **161**, 27-32 (2003).
7. Alpin, A.E., et al., Integrin-mediated adhesion regulates ERK nuclear translocation and phosphorylation of Elk1., *J. Cell Biol.*, **153**, 273-281 (2001).
8. Lee, S.E., et al., Tumor necrosis factor- $\alpha$  supports the survival of osteoclasts through the activation of Akt and ERK., *J. Biol. Chem.* **276**, 49343-49349 (2001).
9. Slack-Davis, J.K., et al., PAK1 phosphorylation of MEK1 regulates fibronectin-stimulated MAPK activation., *Cell Biol.* **162**, 281-291 (2003).
10. Soderstrom, T.S., et al., Mitogen-activated protein kinase/ extracellular signal-regulated kinase signaling in activated T cell abrogates TRAIL-induced apoptosis upstream of the mitochondrial amplification loop and caspase-8., *J. Immunol.* **169**, 2851-2856 (2002)

Triton is a registered trademark of Dow Chemical.

## References

1. Burack, W.R. and T.W. Sturgill, The activating dual phosphorylation of MAPK by MEK is nonprocessive., *Biochemistry*, **36**, 5929-5933 (1997).
2. Mansour, S.J., Interdependent domains controlling the enzymatic activity of mitogen-activated protein kinase kinase 1., *Biochemistry*, **35**, 15529-15536 (1996).
3. Xu, B.E., Hydrophobic as well as charged residues in both MEK1 and ERK2 are important for their proper docking., *J. Biol. Chem.*, **276**, 26509-26515 (2001).

AH,PHC 12/05-1

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.