ELISA PRODUCT INFORMATION & MANUAL

MEK1 [p Ser222, p Ser218]

NBP2-62159

Enzyme-linked Immunosorbent Assay for quantitative detection of Human, Mouse, Rat MEK1.

For research use only.

Not for diagnostic or therapeutic procedures.

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MEK1 [p Ser222, p Ser218] ELISA kit

Catalog No. NBP2-62159

96 Well Kit

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Description

The MEK1 [p Ser222, p Ser218] ELISA kit is a complete kit for the quantitative determination of pMEK 1 in cell lysates. Please read the complete kit insert before performing this assay. The kit uses a monoclonal antibody to MEK 1 immobilized on a microtiter plate to bind the pMEK 1 in the standards or samples. A recombinant phosphorylated MEK 1 Standard is provided in the kit. After a short incubation, the excess sample or standard is washed out and a rabbit polyclonal antibody to pMEK 1/2 is added. This antibody binds to the pMEK 1 captured on the plate. After a short incubation, the excess antibody is washed out and anti-rabbit IgG conjugated to Horseradish peroxidase is added, which binds to the polyclonal pMEK 1/2 antibody. Excess conjugate is washed out and substrate is added. After a short incubation, the enzyme reaction is stopped and the color generated is read at 450 nm. The measured optical density is directly proportional to the concentration of pMEK 1 in either standards or samples. For further explanation of the principles and practices of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

Introduction

MEK1 is also known by a variety of other names. They include dual specificity mitogen-activated protein kinase (MAP kinase kinase 1 or MAPKK 1), ERK activator kinase 1, MAPK/ERK kinase 1, ERK kinase 1 and MAP kinase kinase. MEK1 is a 393 amino acid, 43.5kD protein that is highly conserved in evolution[3]. MEK phosphorylates threonine and tyrosine residues on MAP kinases ERK 1 and 2 (p44 and p42 MAP kinase)[4]. MEK participates in a wide range of cellular processes including cell proliferation[5], differentiation[6] and apoptosis[7]. MEK 1/2 is activated by phosphorylation of Ser^{218} and Ser^{222} by the serine-threonine kinase RAF1, which is part of the p21ras signal transduction pathway. Constitutive activation of MEK 1/2 results in cellular transformation. This protein kinase has been reported to be a likely target for pharmacologic intervention in proliferative diseases[8]. Recent literature reviews cover MEK activity in great detail[9-10].

Precautions

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1. Stop Solution 2 is a 1N hydrochloric acid solution. This solution is caustic; care should be taken in use.
2. The activity of the Horseradish peroxidase conjugate is affected by nucleophiles such as azide, cyanide and hydroxylamine.
3. We test this kit's performance with a variety of buffers, however it is possible that high levels of interfering substances may cause variation in assay results.
4. The addition of protease inhibitor cocktail (PIC) to Assay Buffer 22 will reduce the optical density readings of samples and standards.
5. The phospho-MEK 1 Standard provided, should be handled with care because of the known and unknown effects of MEK.
6. The phospho-MEK 1 Standard should be stored at or below -20 °C. Do not repeatedly freeze-thaw.

Materials Supplied

1. **MEK Microtiter Plate, One Plate of 96 Wells**
   A plate using break-apart strips coated with a mouse monoclonal antibody specific to MEK 1.
2. **pMEK Antibody, 10 mL**
   A yellow solution of rabbit polyclonal antibody to pMEK 1 and 2.
3. **Assay Buffer 22 Concentrate, 100 mL**
   100 mM Tris, pH 7.5, 2 mM EDTA, 2 mM EGTA, 5 mM □-glycerophosphate, 20 mM sodium
   pyrophosphate, 0.1% Triton X-100, 0.1% Tween 20 and 0.1% Hydorol M.
4. **pMEK Conjugate, 10 mL**
   A blue solution of anti-rabbit IgG conjugated to Horseradish peroxidase.
5. **Wash Buffer Concentrate, 100 mL**
   Tris buffered saline containing detergents.
6. **phospho-MEK 1 Standards, 2 each**
   Two vials containing 3000 pg each lyophilized recombinant human phospho-MEK 1.
7. **TMB Substrate, 10 mL**
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide.
   Protect from prolonged exposure to light.
8. **Stop Solution 2, 10 mL**
   A 1N solution of hydrochloric acid in water. Keep tightly capped. Caution: **Caustic.**
9. **pMEK Assay Layout Sheet, 1 each**
10. **Plate Sealer, 3 each**

Storage

All components of this kit, **except the Standard**, are stable at 4 °C until the kit's expiration date. The Standard **must** be stored at or below -20 °C.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Phenylmethanesulfonyl Fluoride (PMSF).
3. Activated Sodium Orthovanadate.
4. Precision pipets for volumes between 100 µL and 1,000 µL.
5. Repeater pipet for dispensing 100 µL.
6. Disposable beakers for diluting buffer concentrates.
7. Graduated cylinders.
8. A microplate shaker.
9. Adsorbent paper for blotting.
10. Microplate reader capable of reading at 450 nm, preferably with correction between 570 nm and 590 nm.
11. Graph paper for plotting the standard curve.

Sample Handling

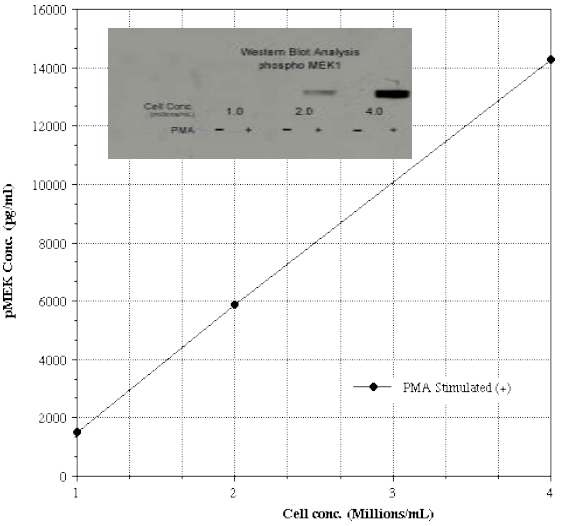
The MEK1 [p Ser222, p Ser218] ELISA is compatible with pMEK 1 samples in a wide range of cell lysates. Samples diluted sufficiently into Assay Buffer 22 plus Inhibitors can be read directly from a standard curve. It is recommended that all samples be lysed with the provided Assay Buffer 22 modified by the addition of 1 mM PMSF and 2 mM activated Sodium Orthovanadate (see Reagent Preparation, page 5, #4) immediately prior to use. Samples lysed with this modified Assay Buffer 22 require no further dilution based on the lysis of 1 million Jurkat cells per mL. However, when working with samples containing more than 1 million Jurkat cells per mL, dilution with Assay Buffer 22 plus Inhibitors is required prior to running the assay. Please refer to the Sample Recovery recommendations on page 11 for details of suggested dilutions.

If the end user chooses to use another lysis buffer, a greater number of cells, or varies from the stimulation procedure noted below, it is up to the end user to determine the appropriate dilution of samples and assay validation. Only standard curves generated in Assay Buffer 22 plus Inhibitors should be used to calculate the concentration of pMEK 1. Samples must be stored frozen at or below –70 °C to avoid loss of bioactive pMEK 1. Excessive freeze/thaw cycles should be avoided. Prior to assay, frozen samples should be brought to 4 °C slowly and gently mixed.

Jurkat Cell Stimulation Experiment

This experiment was adapted from a protocol outlined in reference #11. The number of Jurkat cells used in this experiment were: 4, 2 and 1 million per mL. They were stimulated with 100 nM Phorbol 12-myristate 13-acetate (PMA) or with DMSO (for a negative control) for 10 minutes at 37 °C.

Cells were centrifuged at 1,400 rpm for 5 minutes and the supernatant discarded. The cell pellets were resuspended and washed with PBS. Cells were pelleted at 1,400 rpm for 5 minutes and the supernatant discarded. The cell pellets were resuspended with modified Assay Buffer 22, vortexed and placed at room temperature for 5 minutes. The lysates were vortexed and centrifuged at 1,400 rpm for 5 minutes. The supernatants were split for the Western blot and ELISA to generate the data illustrated. Note that 20 µL of sample was used per lane in the Western blot. The exposure time for development was 20 seconds. The unstimulated cells were below the detection limit of the ELISA.



Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards must be made up in polypropylene tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4 °C in the sealed bag provided. The wells should be used in the frame provided.
8. **Prior to addition of antibody, conjugate and substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.**

Reagent Preparation

1. Wash Buffer

Prepare the Wash Buffer by diluting 50 mL of the supplied concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. Activated Sodium Orthovanadate

Prepare a 200 mM solution of Sodium Orthovanadate. Adjust the pH to 10.0 using either 1N NaOH or 1N HCl (at pH 10.0 the solution will be yellow). Boil the solution till it turns colorless (approximately 10 minutes). Cool the solution to room temperature. Readjust the pH to 10.0. Repeat the boiling and pH readjustment steps until the solution remains colorless and the pH stabilizes at 10.0. Aliquot and store the solution at -20 °C.

3. Assay Buffer 22

Prepare the Assay Buffer 22 by diluting 100 mL of the supplied concentrate with 400 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

4. Assay Buffer 22 plus Inhibitors

Immediately prior to use in assay, add PMSF to a final concentration of 1 mM, and Activated Sodium Orthovandate to a concentration of 2 mM. Ensure Assay Buffer 22 plus Inhibitors is completely in solution prior to use. Do not add PIC to Assay Buffer 22.

This modified Assay Buffer 22 must be used for standard reconstitution and all sample and standard dilutions to ensure optimal integrity of pMEK 1. Fresh Assay Buffer 22 plus Inhibitors must be made for each assay.

5. **phospho-MEK 1 Standards**
   Allow the lyophilized phospho-MEK 1 standard to warm to room temperature. Add 500 µL of Assay Buffer 22 plus Inhibitors to the lyophilized phospho-MEK 1 vial and vortex. Wait 5 minutes and vortex again prior to use. Label the vial Standard #1. Label five 12x75 mm polypropylene tubes #2 through #6. Pipet 250 µL of Assay Buffer 22 plus Inhibitors into tubes #2 through #6. Add 250 µL of vial #1 to tube #2 and vortex thoroughly. Continue this for tubes #3 through #6. **The concentration of pMEK1 in tubes #1 through #6 will be 6,000, 3,000, 1,500, 750, 375, and 187.5 pg/mL respectively. See pMEK 1 Assay Layout Sheet for dilution details. Reconstituted and diluted standards should be used within 20 minutes of preparation.**

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening. All standards, controls and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4 °C.
2. Pipet 100 µL of Assay Buffer 22 plus Inhibitors into the S0 (0 pg/mL standard) wells.
3. Pipet 100 µL of Standards #1 through #6 into the appropriate wells.
4. Pipet 100 µL of the Samples into the appropriate wells.
5. Tap the plate gently to mix the contents.
6. Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
7. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 3 more times for a total of **4 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
8. Pipet 100 µL of yellow Antibody into each well, except the Blank.
9. Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
10. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 3 more times for a total of **4 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
11. Add 100 µL of blue Conjugate to each well, except the Blank.
12. Seal the plate and incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm.
13. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 3 more times for a total of **4 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
14. Pipet 100 µL of Substrate Solution into each well.

15. Incubate for 30 minutes at room temperature on a plate shaker at ~500 rpm.
16. Pipet 100 µL Stop Solution 2 to each well. This stops the reaction and the plate should be read immediately.
17. Blank the plate reader against the Blank wells, read the optical density at 450 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all the readings.

Calculation of Results

Several options are available for the calculation of the concentration of pMEK 1 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of pMEK 1 can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average Blank OD

2. Plot the Average Net OD for each standard versus pMEK 1 concentration in each standard. Approximate a straight line through the points. The concentration of pMEK 1 in the un- knowns can be determined by interpolation.

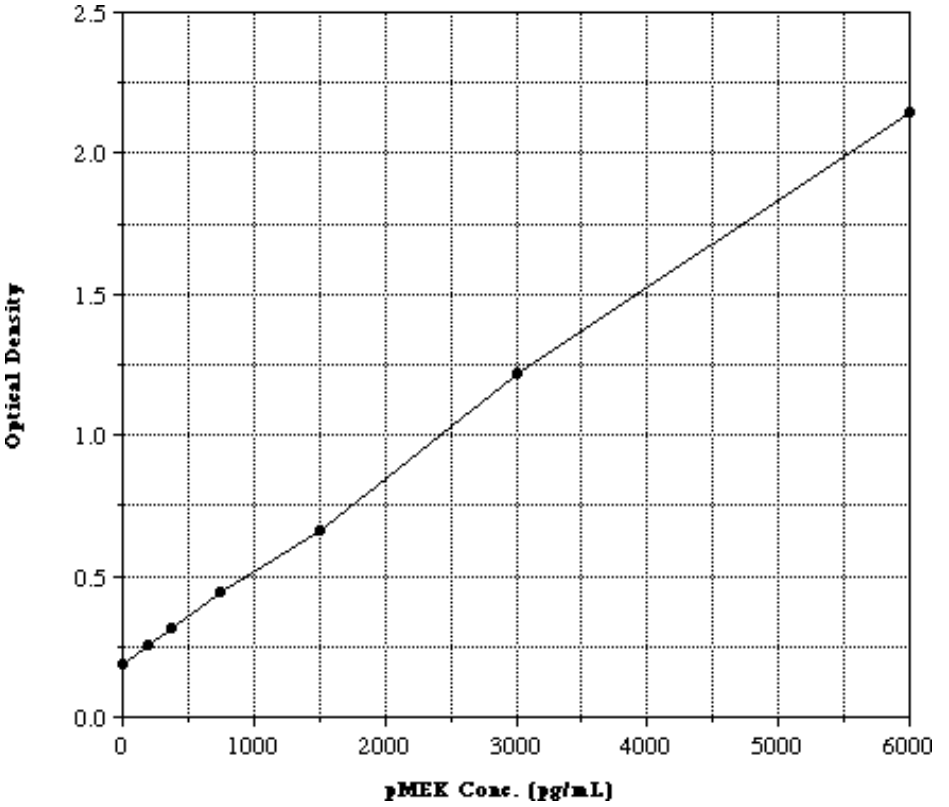
Typical Results

The results shown below are for illustration only and **should not** be used to calculate results from another assay.

Sample	Average OD	Net OD	**pMEK 1 (pg/mL)**
Blank	0.074		
S0	0.261	0.187	**0**
S1	2.220	2.146	**6,000**
S2	1.294	1.220	**3,000**
S3	0.734	0.660	**1,500**
S4	0.519	0.445	**750**
S5	0.390	0.316	**375**
S6	0.328	0.254	**187.5**
Unknown #1	1.630	1.556	**4,062**
Unknown #2	0.377	0.303	**362.9**

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate pMEK 1 concentrations; each user must run a standard curve for each assay.



Units of Measure

Samples measured in the MEK1 [p Ser222, p Ser218] ELISA kit can be expressed in terms of concentration by weight or activity. When reconstituted according to direction, the standard concentration is 6000 pg/mL. To convert this value to Units/mL, the weight concentration is multiplied by the specific activity of the standard. The specific activity of the standard is 8,384 Units/mg where one Unit of pMEK activity equals the maximal activation of one unit of MAP kinase 2/EKK2 per minute at 30 °C. One unit of MAP kinase 2/EKK2 activity is equal to 1 nmole phosphate incorporated into myelin basic protein per minute at 30 °C with a final ATP Concentration of 100 µM.

Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[12].

Sensitivity

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run with 0 pg/mL Standard, and comparing to the average optical density for sixteen (16) wells run with Standard #6. The detection limit was determined as the concentration of pMEK 1 measured at two (2) standard deviations from the 0 pg/mL Standard along the standard curve.

Mean OD for S0 = 0.195 ± 0.010 (4.9%)
Mean OD for Standard #6 = 0.239 ± 0.011 (4.6%)
Delta Optical Density (187.5 - 0 pg/mL) = 0.239- 0.195 = 0.044

2 SD's of 0 pg/mL Standard = 2 x 0.010 = 0.020

$$\text{Sensitivity} = \frac{0.020}{0.044} \times 187.5 \text{ pg/mL} = \textbf{85.2 pg/mL}$$

Linearity

A sample containing 4,940 pg/mL pMEK 1 was serially diluted 4 times 1:2 in the Assay Buffer 22 plus Inhibitors supplied in the kit and measured in the assay. The data was plotted graphically as actual pMEK1 concentration versus measured pMEK 1 concentration.

The line obtained had a slope of 0.963 with a correlation coefficient of 0.998.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of pMEK 1 and running these samples multiple times (n=16) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium, and high concentrations of pMEK 1 in multiple assays over two days (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of pMEK 1 determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	pMEK 1 (pg/mL)	Intra-assay % CV	Inter-assay % CV
Low	369	4.7	
Medium	1,338	4.1	
High	4,043	3.4	
Low	429		14.8
Medium	1,208		7.9
High	3,875		3.9

Cross Reactivities.

The cross reactivities for a number of related compounds were determined by dissolving the cross reactant in the kit assay buffer. These samples were then measured in the pMEK 1 assay and measured pMEK 1 concentration calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

Compound	Cross Reactivity
pMEK 1	100%
pMEK 2	12.2%
MEK 1 (inactive)	<0.1%
pJNK	<0.1%
ERK 2	<0.1%
pERK 2	<0.1%

Sample Recoveries

Please refer to pages 4 and 5 for Sample Handling recommendations and Standard preparation.

pMEK 1 concentrations were measured in cell lysates diluted with Assay Buffer 22 plus Inhibitors and assayed in the kit. The following results were obtained:

Sample	% Recovery*	Recommended Dilution*
1 million Jurkat cells per mL	99.6	none
2 million Jurkat cells per mL	95.2	1:2
4 million Jurkat cells per mL	96.9	1:4

WARNING: If the end user chooses to not use the provided Assay Buffer 22 plus Inhibitors, it is up the end user to determine the appropriate dilution of samples and assay validation for their chosen cell lysis buffer.

* See Sample Handling instructions on page 4 for details.

References

1. T. Chard, "An Introduction to Radioimmunoassay & Related Techniques 4th Ed.", (1990) Amsterdam: Elsevier.
2. P. Tijssen, "Practice & Theory of Enzyme Immunoassays", (1985) Amsterdam: Elsevier.
3. C.M. Crews, et al., Science, (1992) 258:478-480.
4. G. Hardie and S. Hanks,"The Protein Kinase Fact Book, Protein-Serine Kinases", (1995) San Diego, CA: Academic Press. 418.
5. C.C. Lin, et al., Cell Signal, (2002) 14(3):265-75.
6. M.B. Miranda, et al., Leukemia, (2002) 16(4):683-92.
7. H. Lin, et al., Exp. Cell Res., (2002) 272(2):192-8.
8. J.S. Sebolt-Leopold, et al., Nat. Med., (1999) 5(7):192-8.
9. J.T. Lee, et al., Leukemia, (2002) 16(4):485-507.
10. C. Peyssonnaux and A. Eychene, Biol. Cell., (2001) 93(1-2):53-62.
11. T.W. Schulte, et al., Mol. Cell. Biol., (1996) 16(10):5839-45.
12. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

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