Total MEK1/2 ELISA Kit

Catalog Number EMS2MEKT

Product description

An immunoassay for the quantitative determination of pan or total MEK1/2 in cell lysates. Please read the complete kit insert before performing this assay. The kit uses a monoclonal antibody to MEK1 immobilized on a microtiter plate to bind the MEK1 in the standards or samples. A recombinant phosphorylated MEK1 Standard is provided in the kit.

Contents and storage

The kit and components are shipped at -20°C. Upon receipt, store the Total MEK1/2 ELISA Kit at 4°C.

Total MEK1/2 ELISA Kit (EMS2MEKT1)	Amount
Antibody coated 96-well plate	1 plate
Reagent Diluent Concentrate	100 mL
Total MEK Antibody	10 mL
Goat anti-rabbit-HRP Conjugate	10 mL
Cell Lysis Buffer	100 mL
20X Wash Buffer	100 mL
TMB Substrate	10 mL
Stop Solution (1N HCl)	10 mL
Plate Sealer	3 each

Store the Total MEK1/2 Standard at –20°C or lower. Avoid repeated freeze-thaw cycles.

Total MEK1/2 Standard (EMS2MEKT2)	Amount
Total MEK1/2 Standard (5,000 pg each; lyophilized recombinant human phosphorylated MEK1)	2 vials

Additional required materials

- Deionized or distilled water
- Phenylmethylsulfonyl fluoride (PMSF)
- Protease Inhibitor Cocktail (PIC) (Sigma #P8340 or equivalent)
- Activated sodium orthovanadate
- Precision pipettes (for volumes between 100 µL and 1,000 µL)
- Repeater pipettes (for dispensing 100 µL)
- Disposable beaker for diluting buffer concentrates
- 12 × 75 mm polypropylene tubes
- Graduated cylinders
- Microplate shaker
- Absorbent paper for blotting
- Microplate reader capable of reading at 450 nm

General guidelines

- Do not mix components from different kit lots or use reagents beyond the kit expiration date.
- Make standards in polypropylene tubes.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard or reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the well to avoid contamination.
- Allow kit components to come to room temperature for at least 30 minutes before use.
- 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.

Assay compatibility

The MEK1/2 ELISA Kit is compatible with MEK samples in a wide range of cell lysates.

Reagent Preparation

Activated Sodium Orthovanadate

Prepare a 200 mM solution of Sodium Orthovanadate using Sigma #S6508 or equivalent. Adjust the pH to 10 using either 1N NaOH or 1N HCl (at pH 10, solution is yellow). Boil the solution until it turns colorless (~10 minutes). Cool the solution to room temperature. Readjust the pH to 10. Repeat the boiling and pH readjustment steps until the solution remains colorless and the pH stabilizes at 10. Aliquot and store the solution at -20°C.

Cell Lysis Buffer Plus Inhibitors

Allow to come to room temperature. Ensure Cell Lysis Buffer is completely in solution prior to use. Immediately prior to cell lysis, PMSF and Activated Sodium Orthovanadate must be added to the buffer. Add PMSF, such as Sigma #P7626, to a final concentration of 1 mM. Add Activated Sodium Orthovanadate to a final concentration of 2 mM. Do not add Protease Inhibitor Cocktail (PIC) to this buffer if the same lysates will be used in the MEK1/2 [pS218/pS222] ELISA kit (Catalog No. EMSMEKP). Fresh Cell Lysis Buffer Plus Inhibitors must be made each time the cells are lysed.

Reagent Diluent Plus Inhibitors

Immediately prior to use in the assay, PMSF and PIC must be added to the buffer. If using Sigma Protease Inhibitor Cocktail #P8340, add 0.5 µL/mL or equivalent concentration according to alternate vendor's specification sheet. Add PMSF, such as Sigma #P7626, to a final concentration of 1 mM.
This modified Reagent Diluent must be used for standard reconstitution and all sample and standard dilutions to ensure optimal integrity of MEK1/2. Fresh Reagent Diluent Plus Inhibitors must be made for each assay.

Prepare 1X Wash Buffer

1. Allow the 20X Wash Buffer to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 50 mL of Wash Buffer Concentrate with 950 mL of deionized or distilled water. Label as 1X Wash Buffer.

   The diluted buffer is stable for up to 3 months at room temperature.

Sample handling

- Store samples at –80°C to avoid loss of bioactive MEK1.
- Avoid excessive freeze-thaw cycles.
- Slowly warm frozen samples to 2-8°C with gentle mixing prior to assay.

Sample preparation guidelines

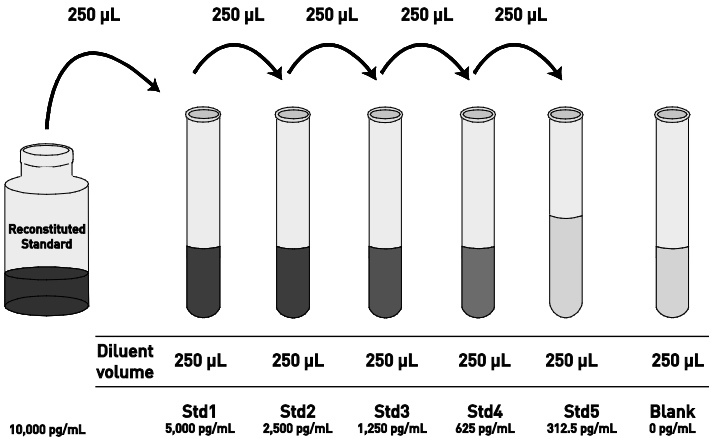
- Samples lysed with Cell Lysis Buffer Plus Inhibitors require further dilution with Reagent Diluent Plus Inhibitors prior to running the assay.
- A minimum 1:4 to 1:16 dilution is recommended to remove matrix interference in the assay.
- Samples diluted sufficiently into Reagent Diluent Plus Inhibitors can be read directly from a standard curve.
- If using other lysis buffers, determination of an appropriate dilution for samples and assay validation is required.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Standard preparation guidelines

- Only standard curves generated in Reagent Diluent Plus Inhibitors should be used to calculate the concentration of MEK1/2.
- Use the reconstituted and diluted standards within 20 minutes.

Dilute standards

1. Add 500 µL Reagent Diluent Plus Inhibitors to the lyophilized Total MEK1/2 Standard vial and vortex. Label as 10,000 pg/mL MEK1.
2. Add 250 µL reconstituted standard to one tube containing 250 µL of Reagent Diluent Plus Inhibitors. Label as 5,000 pg/mL MEK1.
3. Add 250 µL of Reagent Diluent Plus Inhibitors to each of 5 tubes labeled as follows: 2,500; 1,250; 625; 312.5; and 0 pg/mL MEK1.
4. Discard all remaining reconstituted and diluted standards after completing assay.



ELISA procedure

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2-8°C for future use.

Run all standards and samples in duplicate.

1. Add 100 µL of Reagent Diluent Plus Inhibitors into the S0 (0 pg/mL Standard) wells.
2. Add 100 µL of Standards #1 through #6 into the appropriate wells.
3. Add 100 µL of the Samples into the appropriate wells.
4. Seal the plate and incubate at room temperature on a plate shaker (~500 rpm) for 1 hour.
5. Empty the contents of the wells and wash by adding 400 µL of the 1X Wash Buffer to every well. Repeat the wash 3 more times for a total of 4 washes.
6. 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.
7. Add 100 µL of the yellow Total MEK Antibody into each well, except the Blank well.
8. Seal the plate and incubate at room temperature on a plate shaker (~500 rpm) for 1 hour.
9. Empty the contents of the wells and wash by adding 400 µL of the 1X Wash Buffer to every well. Repeat the wash 3 more times for a total of 4 washes.
10. 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 the blue goat anti-rabbit IgG-HRP Conjugate into each well, except the Blank well.
12. Seal the plate and incubate at room temperature on a plate shaker (~500 rpm) for 30 minutes.
13. Empty the contents of the wells and wash by adding 400 µL of the 1X Wash Buffer 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. Add 100 µL of the TMB Substrate Solution to every well. Incubate at room temperature on a plate shaker (~500 rpm) for 30 minutes.
15. Add 100 µL of Stop Solution to every well and read the plate immediately.
16. Blank the plate reader against the Blank wells, read the optical density at 450 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 readings.

Calculations

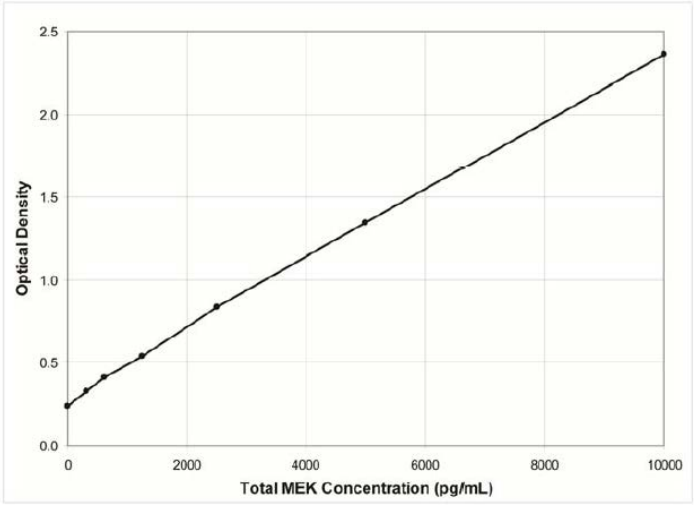
Several options are available for the calculation of the concentration of total MEK1/2 in the samples. It is recommended that the data be analyzed by a 4-parameter logistic curve fitting program. If data reduction software is not available, the concentration of total MEK1/2 can be calculated as follows:

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. Using linear graph paper, plot the Average Net OD for each standard versus total MEK1/2 concentration in each standard. Approximate a straight line through the points. The concentration of MEK in the unknowns can be determined by interpolation.

Typical standard curve

A typical standard curve is shown below. This curve must not be used to calculate total MEK concentrations; a standard curve must be run with every assay.



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.

Sensitivity

Sensitivity of the assay, defined as the concentration of Total MEK measured at 2 standard deviations from the mean of 20 zeros along the standard curve, was determined to be 139.0 pg/mL.

Spike and Recovery

After diluting each sample matrix to its minimum required dilution, recombinant Total MEK1/2 was spiked at high, medium, and low concentrations. The recovery of the standard in spiked samples was compared to the recovery of identical spikes in the assay buffer. The mean and range of percent at three concentrations are indicated below for each matrix.

Sample Matrix	MRD	Unique Samples	Spike Concentration (pg/mL)	Spike Recovery
Jurkat lysates treated with PMA	1:4	1	7000	103%
			1700	99%
			400	121%
Jurkat lysates treated with PMA	1:8	1	7000	104%
			1700	100%
			400	98%
Jurkat lysates treated with DMSO	1:4	1	7000	83%
			1700	84%
			400	104%
Jurkat lysates treated with DMSO	1:8	1	7000	90%
			1700	86%
			400	117%

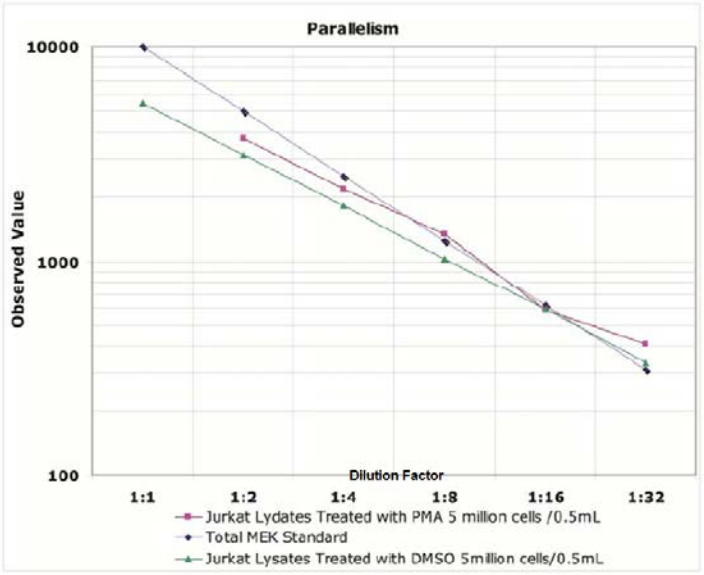
Linearity

The minimum required dilution for several common samples was determined by serially diluting samples into the assay buffer plus inhibitors and identifying the dilution at which linearity is observed. Jurkat lysates treated with PMA and DMSO were diluted in to the assay buffer plus inhibitors to produce values within the dynamic range of the assay.

	Average % of Expected	
Dilution	Jurkat lysates treated with PMA 5M cells/0.5mL	Jurkat lysates treated with DMSO 5M cells/0.5mL
1:2	58%	51%
1:4	67%	58%
1:8	82%	68%
1:16	73%	76%
1:32	100%	89%
1:64	—	100%

Parallelism

A parallelism experiment was carried out to determine if the recombinant total MEK1/2 standard accurately measures MEK concentrations in biological matrices. To assess parallelism, values for Jurkat lysates were obtained from standard curve using four-parameter logistic curve fitting. The obtained concentrations were plotted against the dilution factor. Parallelism indicates antibody-binding characteristics of the native and standard proteins are similar, allowing accurate determination of analyte.



Precision

Intra-assay precision was determined by taking by assaying 20 replicates of three buffer controls containing Total MEK in a single assay. Inter-assay precision was determined by measuring buffer controls (n=20) of varying Total MEK concentrations in multiple assays over several days.

Intra-assay	Total MEK1/2 (pg/mL)	%CV
Low	1477	5.4
Medium	779	5.6
High	385	15.7

Inter-assay	Total MEK1/2 (pg/mL)	%CV
Low	1457	6.1
Medium	796	11.9
High	432	5.9

Cross-reactivity

The cross reactivities of a number of related compounds were determined by dissolving the cross reactant in the kit assay buffer. These samples were then measured in the Total MEK1/2 assay and measured total MEK1/2 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
pMEK1	91.0%
pMEK2	17.4%
pJNK	5.7%
ERK2	0.4%
GSK-3α	0.1%
p38	0.1%

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use	Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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