MEK1/2 [pS218/pS222] ELISA Kit

Catalog Number: EMSMEKP

Pub. No. MAN0014048 **Rev** 2.0

Product description

An immunoassay for the quantitative determination of MEK1/2 dual-phosphorylated at serine 218 and serine 222 in cell lysates.

Contents and storage

The kit and components are shipped at -20°C. Upon receipt, store the MEK1/2 [pS218/pS222] ELISA Kit at 4°C.

MEK1/2 [pS218/pS222] ELISA Kit (EMSMEKP1)	Amount
MEK1 Precoated 96-well Strip Plate	1 plate
Reagent Diluent Concentrate	100 mL
MEK1/2 [pS218/pS222] Detection Antibody	10 mL
Goat anti-rabbit-HRP Conjugate	10 mL
20X Wash Buffer	100 mL
TMB Substrate Solution	10 mL
Stop Solution (1N HCl)	10 mL
Plate Sealer	3 each

Store the MEK1/2 [pS218/pS222] Standard at –20°C or lower. Avoid repeated freeze-thaw cycles.

MEK1/2 [pS218/pS222] Standard (EMSMEKP2)	Amount
MEK1/2 [pS218/pS222] Standard (3,000 pg recombinant human phospho-MEK1, lyophilized)	2 vials

Additional required materials

- Deionized or distilled water
- Phenylmethylsulfonyl fluoride (PMSF)
- 200 mM Sodium orthovanadate, pH 10.0
- 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 lint free paper for blotting
- Microplate reader capable of reading at 405 nm, preferable with correction between 570 and 590 nm.

General guidelines

- Do not mix components from different kit lots or use reagents beyond the kit expiration date.
- 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 substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.

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Assay compatibility

The MEK 1/2 [pS218/pS222] ELISA Kit is compatible with phosphorylated MEK samples in a wide range of matrices after dilution in Reagent Diluent Plus Inhibitors.

Prepare 1X Reagent Diluent

Dilute 100 mL of Reagent Diluent Concentrate with 400 mL of deionized or distilled water. Label as 1X Reagent Diluent.

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

Prepare Reagent Diluent Plus Inhibitors

Use the Reagent Diluent Plus Inhibitors for MEK1/2 [pS218/pS222] Standard reconstitution and all sample and standard dilutions to ensure optimal integrity of phosphorylated MEK.

Prepare fresh Reagent Diluent Plus Inhibitors for each assay by adding inhibitors to 1X Reagent Diluent at the final concentration listed in the following table. Do not add PIC. Ensure Reagent Diluent Plus Inhibitors is completely in solution prior to use.

Inhibitor	Final concentration
PMSF	1 mM
Activated Sodium Orthovanadate	2 mM

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 20X Wash Buffer 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 –70°C to avoid loss of bioactive pMEK.
- Avoid excessive freeze-thaw cycles.
- Slowly warm frozen samples to 2°C to 8°C and mix gently prior to assay.

Sample preparation guidelines

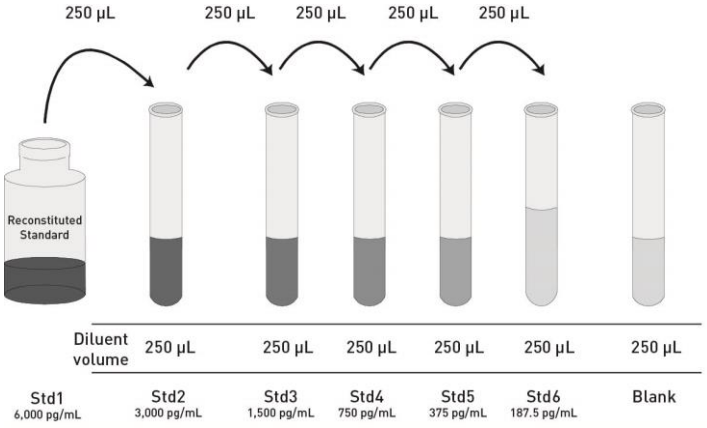
- Lyse samples with fresh Reagent Diluent Plus Inhibitors immediately before use.
- Samples lysed in fresh Reagent Diluent Plus Inhibitors require no further dilution (based on the lysis of 1×10^6 Jurkat cells/mL).
- For more concentrated samples, dilute with Reagent Diluent Plus Inhibitors prior to running the assay.
- 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 fresh Reagent Diluent Plus Inhibitors should be used to calculate the concentration of MEK1/2 [pS218/pS222].
- Use the diluted standards within 20 minutes of preparation.

Reconstitute and dilute standards

1. Add 0.5 mL fresh Reagent Diluent Plus Inhibitors to the lyophilized MEK1/2 [pS218/pS222] Standard vial and vortex.
2. Wait for 5 minutes and vortex again prior to use.
3. Label the reconstituted Standard as vial #1 and label five additional 12 x 75 mm polypropylene tubes #2 through #6
4. Add 250 µL Reagent Diluent Plus Inhibitors to Tubes #2 to #6.
5. Add 250 µL Reconstituted MEK1/2 [pS218/pS222] Standard to Tube #2 and vortex thoroughly.
6. Make serial dilutions of the standard as shown in the dilution diagram below.



ELISA procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening. 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 to 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 MEK1/2 [pS218/pS222] Detection 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-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.
14. 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.
15. Add 100 µL of the TMB Substrate Solution to every well. Seal the plate and incubate at room temperature on a plate shaker (~500 rpm) for 30 minutes.
16. Add 100 µL of Stop Solution to every well and read the plate immediately.
17. Blank the plate reader against the Blank wells and 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 readings.

Calculations

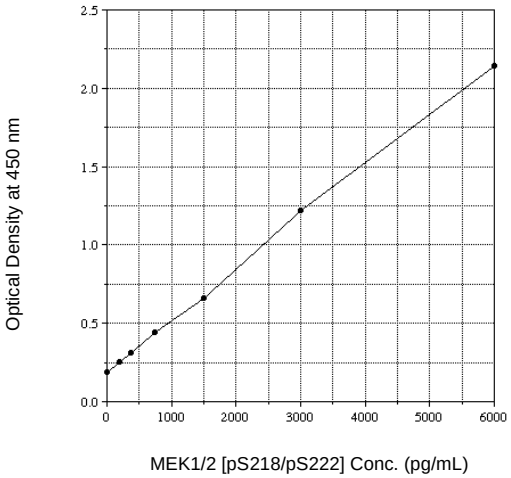
Several options are available for the calculation of the concentration of MEK1/2 [pS218/pS222] in the samples. It is recommended that the data be analyzed by a 4 parameter logistic curve fitting program. If data reduction software is not available, the concentration of MEK1/2 [pS218/pS222]can be calculated 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 MEK1/2 [pS218/pS222] concentration in each standard. Approximate a straight line through the points. The concentration of MEK1/2 [pS218/pS222] 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 MEK1/2 [pS218/pS222] 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

The minimum detectable amount of MEK1/2 [pS218/pS222] is 85.2 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained from the average OD bound for 16 wells run as S0, compared to the average OD for 16 wells run with Standard #6. The detection limit was determined as the concentration of pMEK 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 × 0.010 = 0.020

Sensitivity = (0.020/0.044) × 187.5 pg/mL = 85.2 pg/mL

Linearity

A sample containing 4,940 pg/mL MEK1/2 [pS218/pS222] was serially diluted in Reagent Buffer Plus Inhibitors over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a line with a slope of 0.963 and

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of MEK1/2 [pS218/pS222] and running these samples multiple times (n=16) in the same assay. Inter-assay precision was determined by measuring 2 samples with low and high concentrations of MEK1/2 [pS218/pS222] in multiple assays (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of MEK1/2 [pS218/pS222] determined in these assays as calculated by a 4 parameter logistic curve-fitting program.

Intra-assay	MEK1/2 [pS218/pS222] (pg/mL)	%CV
Low	369	4.7
Medium	1,338	4.1
High	4,043	3.4

Inter-assay	MEK1/2 [pS218/pS222] (pg/mL)	%CV
Low	429	14.8
Medium	1,208	7.9
High	3,875	3.9

Cross-reactivity

The MEK1/2 [pS218/pS222] ELISA Kit is specific for phosphorylated MEK1/2.

Compound	Cross Reactivity
pMEK-1	100%
pMEK-2	12.2%
MEK-1 (inactive), pJNK, ERK2, pERK2	<0.01%

Sample recovery

MEK1/2 [pS218/pS222] concentrations were measured in cell lysates diluted with Reagent Buffer Plus Inhibitors.

Sample	% Recovery	Recommended Dilution
1 x 10^6 Jurkat cells/mL	99.6	None
2 x 10^6 Jurkat cells/mL	95.2	1:2
4 x 10^6 Jurkat cells/mL	96.9	1:4

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