

Version v4a Last updated 25 November 2024

# ab126448 Mek1 (pS217/221) + total Mek1 ELISA Kit

For the semi-quantitative measurement of Human, Mouse and Rat phosphorylated Mek1 (Ser217/221) + total Mek1 concentrations in cell lysates.

This product is for research use only and is not intended for diagnostic use.

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# 1. Overview

ab126448 is a very rapid, convenient and sensitive assay kit that can monitor the activation or function of important biological pathways in cell lysates. By determining phosphorylated Mek1 protein in your experimental model system, you can verify pathway activation in your cell lysates. You can simultaneously measure numerous different cell lysates without spending excess time and effort in performing a Western Blotting analysis.

This sandwich ELISA kit is an in vitro enzyme-linked immunosorbent assay for the measurement of phospho-Mek1 (Ser217/221) and total Mek1 in human, mouse and rat cell lysates (help normalize the results of phospho-Mek1 from different cell lysate being compared). An anti-pan-Mek1 antibody has been coated onto a 96-well plate. Samples are pipetted into the wells and Mek1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and anti-Mek1 (Ser217/221) or anti-pan-Mek1 antibody is used to detect phosphorylated Mek1 (Ser217/221) or total Mek1. After washing away unbound antibody, HRP-conjugated anti-rabbit IgG is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Mek1 (Ser217/221) or total Mek1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

## 2. Protocol Summary

Prepare all reagents, samples, and Positive Controls dilutions as instructed



Add 100  $\mu$ L Positive Control or Sample to each well. Incubate 2.5 hours at room temperature or overnight at 4°C.



Wash the plate.



Add 100  $\mu$ L Detection Antibody Mek1 and Detection Antibody Mek1 (Ser217/221) to the appropriate wells. Incubate 1 hour at room temperature.



Wash the plate.



Add 100  $\mu$ L 1X HRP-conjugated anti-rabbit IgG concentrate. Incubate overnight at 4°C.



Wash the plate.



Add 100  $\mu$ L TMB One-Step Substrate Reagent to each well. Incubate 30 minutes in the dark at room temperature.



Add 50  $\mu$ L Stop Solution to each well. Read at 450 nm immediately.

### 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All ELISA kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

### 4. Storage and Stability

**Store ELISA kit at -20°C immediately upon receipt. Kit has a storage time of 6 months from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

## 5. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

Item	Quantity	Storage Condition	Storage after first use / preparation
Mek1 Microplate (12 strips x 8 wells) coated with anti-pan Mek1 antibody	1 unit	-20°C	-20°C
20X Wash Buffer Concentrate	25 mL	-20°C	4°C
Positive Control: lyophilized powder from HeLa cell lysate	1 vial	-20°C	-70°C
Detection Antibody Mek1	1 vial	-20°C	4°C / -70°C
Detection Antibody Mek1 (Ser217/221)	1 vial	-20°C	4°C / -70°C
500X HRP-conjugated anti-rabbit IgG	25 µL	-20°C	Do not store once diluted
TMB One-Step Substrate Reagent	12 mL	-20°C	4°C
Stop Solution	8 mL	-20°C	4°C
5X Assay Diluent	15 mL	-20°C	4°C
2X Cell Lysis Buffer	5 mL	-20°C	4°C

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Protease and Phosphatase inhibitors.
- Shaker.
- Precision pipettes to deliver 2  $\mu$ l to 1 ml volumes.
- Adjustable 1-25 ml pipettes for reagent preparation.
- 100 mL and 1 L graduated cylinders.
- Distilled or deionized water.
- Tubes to prepare sample dilutions.

## 8. Technical Hints

- Samples generating values higher than the most concentrated Positive Control dilution sample should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, Positive Control and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- When generating positive control dilutions, it is advisable to change pipette tips after each step.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or Positive Control solution will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

## 9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

### 9.1 20X Wash Buffer Concentrate:

If the 20X Wash Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

### 9.2 5X Assay Diluent:

Dilute 5X Assay Diluent 5-fold with deionized or distilled water before use.

### 9.3 Positive Control:

Dilute Briefly spin the Positive Control Vial. Add 500  $\mu$ L of prepared 1X Assay Diluent to the vial to prepare a Positive Control Stock Solution. Gently mix the powder to allow it to dissolve thoroughly. If a precipitate is seen in the solution after mixing, this can be removed by a quick centrifuge of the positive control vial, and then pipetting the supernatant only for the assay. Store for up to 1 week at -80°C.

### 9.4 Detection Antibody Mek1:

Add 100  $\mu$ L of 1x Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days or at -80°C for one month). The antibody should be diluted 55-fold with 1x Assay Diluent and used in Step 12.4 of the Assay Procedure.

### 9.5 Detection Antibody Mek1 (Ser217/221):

Add 100  $\mu$ L of 1x Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days or at -80°C for one month). The antibody should be diluted 55-fold with 1x Assay Diluent and used in Step 12.4 of the Assay Procedure.

**9.6 500X HRP-conjugated anti-rabbit IgG concentrate:**

Briefly spin the vial of HRP-conjugated anti-rabbit IgG concentrate before use. HRP-conjugated anti-rabbit IgG should be diluted 500-fold with 1X Assay Diluent and used in Step 12.6 of the Assay Procedure.

**9.7 2X Lysis Buffer:**

Dilute 2-fold with deionized or distilled water. We also recommend the addition of protease and phosphatase inhibitors to the 1X Lysis Buffer prior to use.

All other reagents are ready to use as supplied.

Return unused wells to the pouch containing desiccant pack, reseal along entire edge and store at -20°C.

## 10. Positive Control Preparation

- Always prepare a fresh set of positive control dilutions for every use.
- Discard working positive control dilutions after use as they do not store well.
- The following section describes the preparation of a positive control curve for duplicate measurements (recommended).

**10.1** To prepare positive control dilutions, label 6 tubes with #1 - #6.

**10.2** Add 500  $\mu\text{L}$  of the prepared Positive Control Stock Solution (See Step 9.3) into tube #1.

**10.3** Pipette 270  $\mu\text{L}$  1X Assay Diluent into each of tubes #2 - #6.

**10.4** Use the Positive Control Stock Solution to produce a dilution series (see Table, below).

**10.5** Tube #6 contains no protein and is the Blank control.

Tube #	Volume to dilute	Volume of Assay Diluent
1	500 $\mu\text{L}$ of Positive Control Stock Solution	-
2	30 $\mu\text{L}$ of tube #1	270 $\mu\text{L}$
3	30 $\mu\text{L}$ of tube #2	270 $\mu\text{L}$
4	30 $\mu\text{L}$ of tube #3	270 $\mu\text{L}$
5	30 $\mu\text{L}$ of tube #4	270 $\mu\text{L}$
6 (Control)	-	270 $\mu\text{L}$

## 11. Sample Preparation

Cell lysates:

- Rinse the cells with PBS, making sure to remove any remaining PBS before adding the lysis buffer.
- Solubilize cells at  $4 \times 10^7$  cells/mL in 1X Cell Lysis Buffer (Step 9.6). Pipette up & down to resuspend the pellet. Incubate the lysates with shaking at 2-8°C for 30 minutes.
- Microcentrifuge at 13,000 rpm for 10 minutes at 2-8°C and transfer the supernatants into a clean test tube.
- Lysates should be used immediately or aliquoted and stored at -70°C. Avoid repeated freeze-thaw cycles. Thawed lysates should be kept on ice prior to use.

For the initial experiment, we recommend a serial dilution, such as a 5-fold to 50 - fold dilution, for your cell lysates with 1X Assay Diluent (see Step 9.2) before use.

**Δ Note:** Levels of phosphorylated proteins may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

## 12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all positive controls, controls and samples in duplicate.
- Prepare all reagents, working positive control dilutions, and samples as directed in the previous sections.

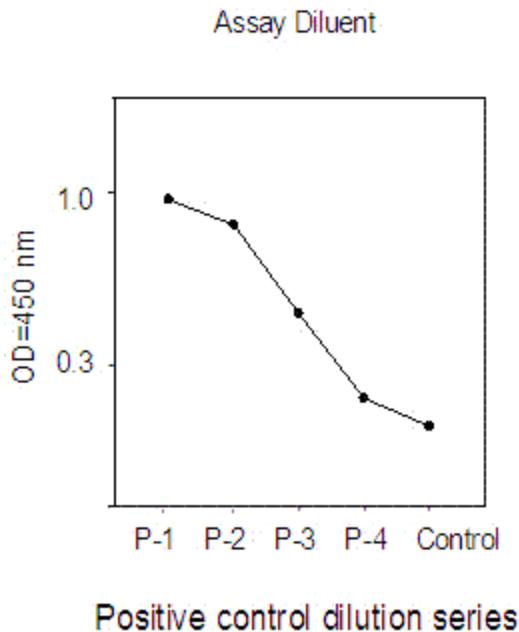
- 12.1** Label removable 8-well strips as appropriate for your experiment.
- 12.2** Add 100  $\mu$ L of Positive Control or Sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.
- 12.3** Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300  $\mu$ L) using a multi-channel Pipette or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
- 12.4** Add 100  $\mu$ L of Detection Antibody Mek1 (Ser217/221) antibody into the wells designated to detect phosphorylated protein. Add 100  $\mu$ L of Detection Antibody Mek1 to the remaining wells to detect total MEK1 protein. Incubate for 1 hour at room temperature with gentle shaking.
- 12.5** Discard the solution. Repeat the wash as in step 12.3.
- 12.6** Add 100  $\mu$ L of HRP-conjugated anti-rabbit IgG to each well. Incubate overnight at 4°C with gentle shaking.
- 12.7** Discard the solution. Repeat the wash as in step 12.3.
- 12.8** Add 100  $\mu$ L of TMB Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
- 12.9** Add 50  $\mu$ L of Stop Solution to each well. Read at 450 nm immediately.

## 13. Calculations

- 13.1 Calculate the mean absorbance for each set of duplicate positive controls, controls and samples.
- 13.2 Subtract the average zero positive control optical density.
- 13.3 Plot the positive control curve on log-log, with positive control concentration on the x-axis and absorbance on the y-axis.

## 14. Typical Data

**Typical Positive Control curve** – data provided for demonstration purposes only. A new positive control curve must be generated for each assay performed.



**Figure 1.** Positive Control Dilution Series.

## 15. Troubleshooting

Problem	Reason	Solution
<b>Poor Positive Control curve</b>	Inaccurate Pipetting	Check pipettes
	Improper positive control dilution	Prior to opening, briefly spin the stock Positive Control tube and dissolve the powder thoroughly by gentle mixing
<b>Low Signal</b>	Improper preparation of Positive Control dilutions and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
	Too brief incubation times	Ensure sufficient incubation time. Sample and Positive Control addition may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background).
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
<b>Large CV</b>	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
<b>High background</b>	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
<b>Low sensitivity</b>	Improper storage of the ELISA kit	Store your Positive Control solution at <-70°C after reconstitution, others at 4°C. Keep substrate solution protected from light.
	Stop solution	Add stop solution to each well before reading plate

## 16. Notes

## Technical Support

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