# RayBio® Human, Mouse and Rat Phospho-MEK1 (Ser217/221) and Total MEK1 ELISA Kit

Catalog #: PEL-Mek-S217-T

User Manual
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Caution:
Extraordinarily useful information enclosed


ISO 13485 Certified

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RayBiotech, Inc.

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Please read the entire manual carefully before starting your experiment

## I. INTRODUCTION

RayBio® Phospho-MEK1 (Ser217/221) and Total MEK1 ELISA kit is a very rapid, convenient and sensitive assay kit that can monitor the activation or function of important biological pathways in Human, Mouse and Rat 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 Human, Mouse and Rat phospho-MEK1 and total MEK1. 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 and the wells are washed. In select wells, rabbit anti-phospho-Mek1 (Ser217/221) antibody is added to detect phosphorylated MEK1. In the remaining wells, rabbit anti-pan-Mek1 antibody is used to detect pan MEK1. After washing away unbound antibody, HRP-conjugated anti-rabbit IgG is pipetted into 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 pan MEK1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

## II. STORAGE

The entire kit may be stored at -20°C for up to 6 months from the date of shipment. Avoid repeated freeze-thaw cycles. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

## III. REAGENTS

| Component | Size / Description | Storage / Stability After Preparation |
|---|---|---|
| Pan MEK1 Microplate (Item A) | 96 wells (12 strips x 8 wells) coated with anti-pan-MEK1. | 1 month at -20°C* |
| Wash Buffer Concentrate (20X) (Item B) | 25 ml of 20X concentrated solution. | 1 month at 4°C |
| Positive Control-HelaT003-1 (Item K) | 1 vial of lyophilized powder from treated Hela cell lysate. | 1 week at -80°C |
| Phospho Detection Antibody MEK1 (Ser217/221) (Item C-1) | 1 vial of rabbit anti-phospho-Mek1 (Ser217/221) (1 vial is enough to assay half of the microplate). | 5 days at 4°C |
| Pan Detection Antibody MEK1 (Item C-2) | 1 vial of rabbit anti-pan-Mek1 (1 vial is enough to assay half microplate). | 5 days at 4°C |
| HRP-conjugated anti-rabbit IgG concentrate (Item D-1) | 1 vial (25 µl) of 500X concentrated HRP-conjugated anti-rabbit IgG. | Do not store and reuse. |
| TMB One-Step Substrate Reagent (Item H) | 12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution. | N/A |
| Stop Solution (Item I) | 8 ml of 0.2 M sulfuric acid. | N/A |
| Assay Diluent (Item E2) | 15 ml of 5X concentrated buffer. For diluting cell lysate samples, detection antibodies (Items C-1 and C-2), and HRP-conjugated anti-rabbit IgG concentrate. | 1 month at 4°C |
| Cell Lysate Buffer (Item J) | 5 ml 2X cell lysis buffer (does not include protease and phosphatase inhibitors). | 1 month at 4°C |

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

## IV. ADDITIONAL MATERIALS REQUIRED

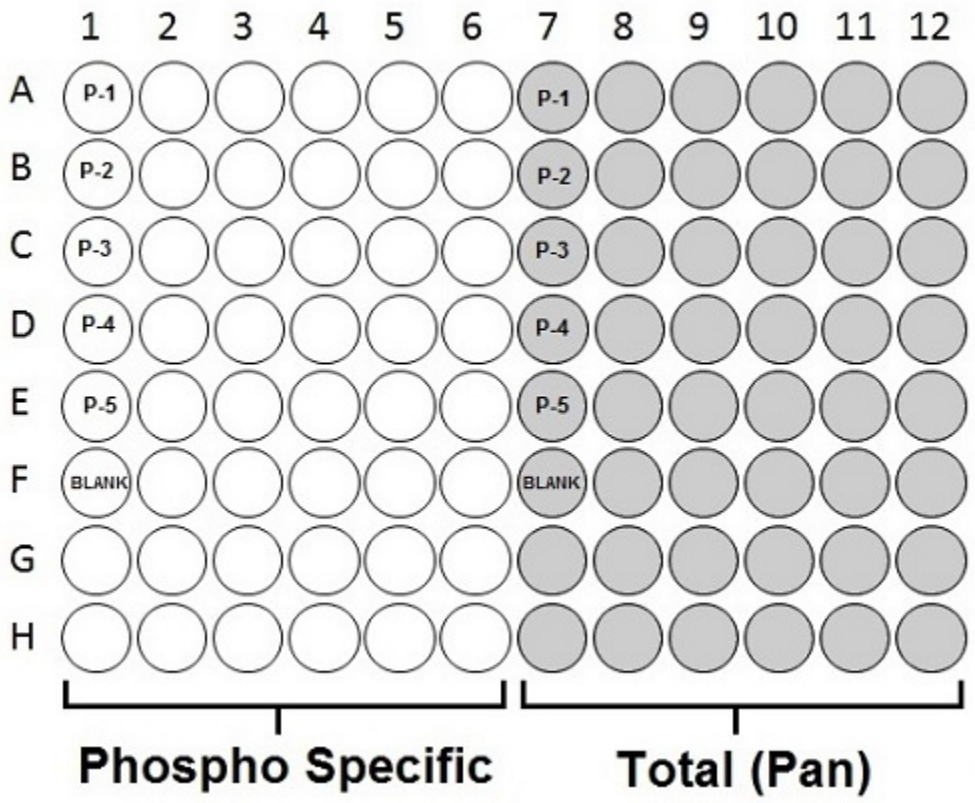
1. Microplate reader capable of measuring absorbance at 450 nm.
2. Protease and Phosphatase inhibitors.
3. Shaker.
4. Precision pipettes to deliver 2 µl to 1 ml volumes.
5. Adjustable 1-25 ml pipettes for reagent preparation.
6. 100 ml and 1 liter graduated cylinders.
7. Absorbent paper.
8. Distilled or deionized water.
9. Log-log graph paper or computer and software for ELISA data analysis.
10. Tubes to prepare the positive control or sample dilutions.

## V. SAMPLE PREPARATION

Cell Lysate Preparation: Rinse the cells with PBS, making sure to remove any remaining PBS before adding the lysis buffer. Solubilize cells at 4 x $10^7$ cells/ml in prepared Cell Lysate Buffer (Item J) (see Reagent Preparation step 3). Pipette up and 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 supernatantes 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 prepared Assay Diluent (Item E2) (see Reagent Preparation step 2) before use.

***Note: The fold dilution of sample used depends on the abundance of phosphorylated proteins and should be determined empirically. More of the sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further.***



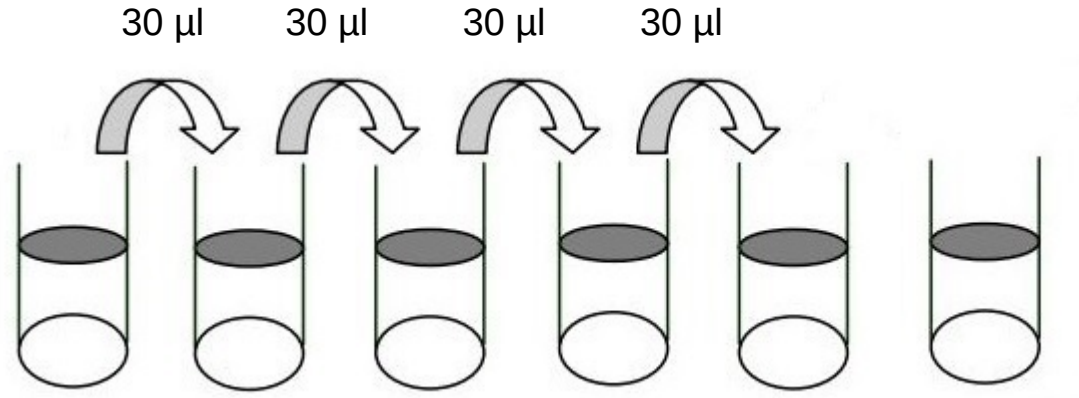
## VI. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use.

2. 5X Assay Diluent (Item E2) should be diluted 5-fold with deionized or distilled water before use.

3. Cell lysate buffer (Item J) should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate). We also recommend the addition of protease and phosphatase inhibitors (not included) to the lysis buffer prior to use.

4. Preparation of Positive Control: Briefly spin the Positive Control Vial (Item K). Add 500 µl of prepared 1X Assay Diluent (Item E2) into Item K to prepare a Positive Control (P-1) 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 supernate only for the assay. Pipette 270 µl 1X Assay Diluent into each tube. Use the Positive Control (P-1) solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Assay Diluent serves as the blank (P-0).




| | P-1 | P-2 | P-3 | P-4 | P-5 | | Blank (P-0) |
|---|---|---|---|---|---|---|---|
| **Diluent volume** | Item K + 500 µl | 270 µl | 270 µl | 270 µl | 270 µl | | 270 µl |

5. If the Wash Concentrate (20X) (Item B) 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.

6. Preparation of Detection Antibodies:

a. Preparation of rabbit anti-phospho-Mek1 (Ser217/221) antibody: Briefly spin the vial of rabbit anti-phospho-Mek1 (Ser217/221) (Item C-1). Add 100 µl of 1X Assay Diluent into the vial to prepare a phospho 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 concentrate should then be diluted 55-fold with 1X Assay Diluent and used in step 4 of the Assay Procedure.
b. Preparation of rabbit anti-pan-Mek1 antibody: Briefly spin the vial of rabbit anti-pan-Mek1 (Item C-2). Add 100 µl of 1X Assay Diluent into the vial to prepare a pan 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 concentrate should then be diluted 55-fold with 1X Assay Diluent and used in step 4 of the Assay Procedure.

7. Preparation of HRP-conjugated anti-rabbit IgG: Briefly spin the vial of HRP-conjugated anti-rabbit IgG concentrate (Item D-1) before use. HRP-conjugated anti-rabbit IgG should be diluted 500-fold with 1X Assay Diluent and used in step 7 of the Assay Procedure.

*For example: Briefly spin the vial (Item D-1) and pipette up and down to mix gently. Add 10 µl of HRP-conjugated anti-rabbit IgG concentrate into a tube with 5 ml 1x Assay Diluent to prepare a 500-fold diluted HRP-conjugated anti-rabbit IgG solution.*

## VII. ASSAY PROCEDURE

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use. It is strongly recommended to run all positive controls and samples in at least duplicate. It is also recommended to run the positive controls in singlet for each of the pan and phospho-specific antibodies.

2. See plate layout (page 5) and label removable 8-well strips as appropriate for your experiment.

3. Add 100 µl of positive control (see Reagent Preparation step 4) or sample into appropriate wells. Cover the wells and incubate for 2.5 hours at room temperature or overnight at 4ºC with gentle shaking.

4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential for 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.

5. Add 100 µl of prepared 1X rabbit anti-phospho-Mek1 (Ser217/221) antibody (see Reagent Preparation step 6a) into the wells designated to detect phosphorylated protein. Add 100 µl of prepared 1X rabbit anti-pan-Mek1 antibody (see Reagent Preparation step 6b) to the remaining wells to detect pan protein. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µl of prepared HRP-conjugated anti-rabbit IgG solution (see Reagent Preparation step 7) to each well. Incubate for overnight at 4°C with gentle shaking.

8. Discard the solution. Repeat the wash as in step 4.

9. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

10. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

## VIII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and positive control as instructed.

2. Add 100 µl positive control or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4ºC with gentle shaking.

3. Add 100 µl prepared detection antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.

4. Add 100 µl prepared HRP-Conjugated solution. Incubate for overnight at 4°C with gentle shaking.

5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

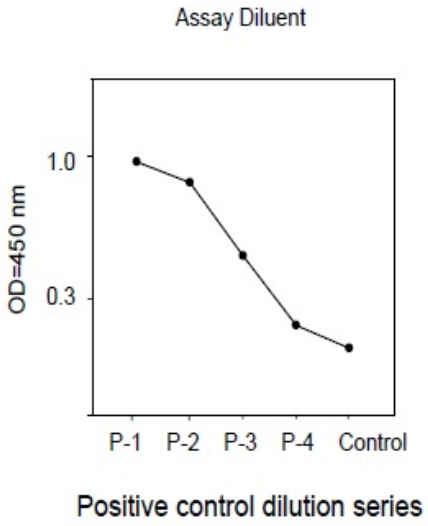
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

## IX. TYPICAL DATA

Calculate the mean absorbance for each sample. Then, subtract the average zero (blank) optical density from each sample mean and set of singlet positive controls.

### A. Positive Control

Hela cells were treated with TPA at 37°C for 15 min. Cells were solubilzed at 4 x $10^7$ cells/ml in Cell Lysate Buffer. Serial dilutions of cell lysates were analyzed in this ELISA (see Reagent Preparation step 4).

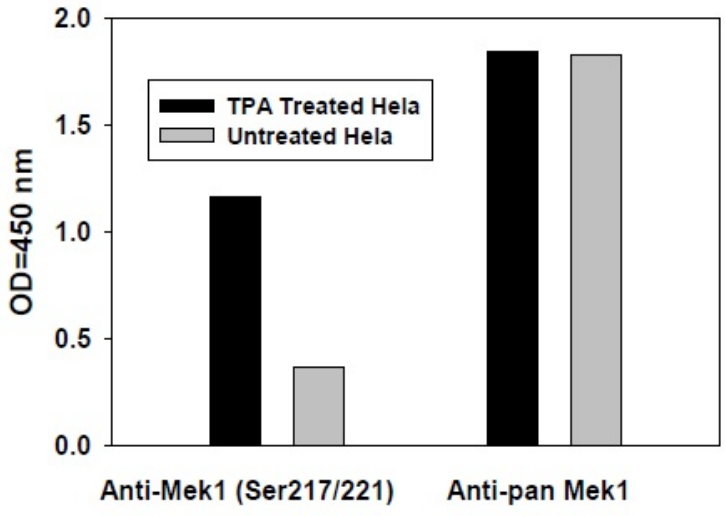



Positive control dilution series

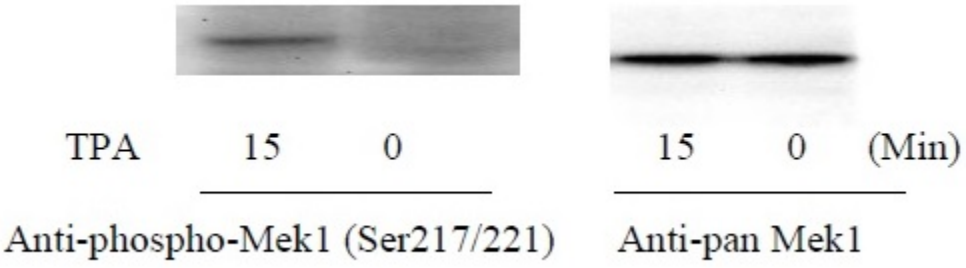
## B. TPA Stimulation of Hela Cell Lines

Hela cells were untreated or treated with TPA for 15 min. Cell lysates were analyzed using this phosphoELISA and Western Blot.

### i. ELISA




### ii. Western-Blot Analysis



## X. TROUBLESHOOTING GUIDE

| Problem | Cause | Solution |
|---|---|---|
| Low signal in samples | • Sample concentration is too low<br>• Improper preparation of detection antibody<br>• Too brief incubation times<br>• Inadequate reagent volumes or improper dilution | • Increase sample concentration<br>• Briefly spin down vials before opening. Dissolve the powder thoroughly.<br>• Ensure sufficient incubation time; assay procedure step 3 may be done overnight<br>• Check pipettes and ensurecorrect preparation |
| High signal in samples | • Sample concentratin is too high | • Reduce sample concentration |
| Large CV | • Inaccurate pipetting<br>• Air bubbles in wells | • Check pipettes<br>• Remove bubbles in wells |
| High background | • Plate is insufficiently washed<br>• Contaminated wash buffer | • Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.<br>• Make fresh wash buffer |
| Low sensitivity | • Improper storage of the ELISA kit<br>• Stop solution<br>• Improper primary or secondary antibody dilution | • Store your positive control at <-70ºC after reconstitution, others at 4ºC. Keep substrate solution protected from light.<br>• Add stop solution to each well before reading plate<br>• Ensure correct dilution |

# RayBio® ELISA Kits

Over 2,000 ELISA kits available, visit www.RayBiotech.com/ELISA-Kits.html for details.

This product is for research use only.