Version 1b Last updated 29 February 2024

ab126453 p38 MAPK alpha (pT180/pY182) + total p38 MAPK alpha ELISA Kit

For the quantitative measurement of human and mouse phosphorylated p38 alpha (Thr180/Tyr182) and total p38 alpha concentrations in cell lysates.

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

Table of Contents

1. Overview

ab126453 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 p38 alpha 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 Blot analysis.

This Sandwich ELISA kit is an in vitro enzyme-linked immunosorbent assay for the measurement of human phospho-p38 alpha (Thr180/Tyr182) and total p38 alpha (help normalize the results of phospho-p38 alpha from different cell lysate being compared). An anti-p38 alpha (Thr180/Tyr182) (half plate, red marker on left side) and anti-total p38 alpha antibody (half plate, black marker on right side) has been coated onto a 96-well plate. Samples are pipetted into the wells and phosphorylated (left side) and total (right side) p38 alpha present in a sample is bound to the wells by the immobilized antibody. The wells are washed and rabbit anti p38 alpha is used to detect phosphorylated or total p38 alpha. 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 p38 alpha (Thr180/Tyr182) or total p38 alpha 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 standards as instructed

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Add 100 µL Positive Control or Sample to appropriate wells (phospho- or total p38 alpha). Incubate 2.5 hours at room temperature or overnight at 4°C.

⇩

Wash the plate.

⇩

Add 100 µL Detection Antibody p38 alpha to each well. Incubate 1 hour at room temperature.

⇩

Wash the plate.

⇩

Add 100 µL 1X HRP-conjugated anti-rabbit IgG concentrate. Incubate for 2 hours at room temperature.

⇩

Wash the plate.

⇩

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

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Add 50 µL 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 handle 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	Starage after first use / reconstitution
p38 alpha Microplate (12 strips x 8 wells)	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 p38 alpha	2 vials	-20°C	4°C / -70°C
500X HRP-conjugated anti-rabbit IgG	25 µL	-20°C	Do not store after dilution
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	10 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 µl 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 highest standard 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, standard 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 samples, 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 standard 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 400 µL 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 p38 alpha:

Add 100 µL 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 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.

9.6 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. Standard Preparation

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

10.1 To prepare standards, label 6 tubes with #1 - #6.
10.2 Add 400 µL of the prepared Positive Control Stock Solution (See Step 9.3) into tube #1.
10.3 Pipette 240 µL 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	400 µL of Positive Control Stock Solution	-
2	120 µL of tube #1	240 µL
3	120 µL of tube #2	240 µL
4	120 µL of tube #3	240 µL
5	120 µL of tube #4	240 µL
6 (Control)	-	240 µL

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 x 10^7 cells/mL in 1X Cell Lysis Buffer (Step 9.6). 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 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 100 - 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 standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

12.1 Label removable 8-well strips as appropriate for your experiment.

Δ Note: Six strips (marked with red marker, provided on the left) are coated with anti-phospho-p38 (Thr180/Tyr182). The remaining 6 strips (marked with black marker, provide on the right) are coated with a pan-p38 antibody.

12.2 Add 100 µL 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 µL) 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 µL of Detection Antibody p38 alpha into each well. 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 µL of HRP-conjugated anti-rabbit IgG to each well. Incubate for 2 hours at room temperature with gentle shaking.

12.7 Discard the solution. Repeat the wash as in step 12.3.

12.8 Add 100 µL of TMB Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

12.9 Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

13. Calculations

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

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

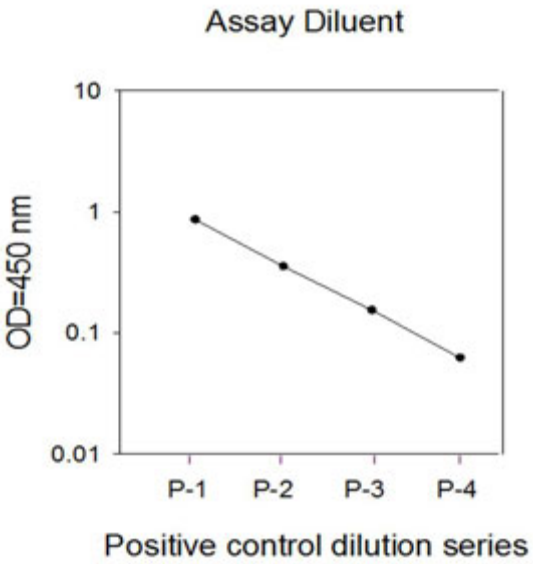

Figure 1. Positive Control Dilution Series.

15. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
	Too brief incubation times	Ensure sufficient incubation time. Sample and standard 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
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
High background	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
Low sensitivity	Improper storage of the ELISA kit	Store your standard 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

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)