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Product Information

Phospho-p38 α (pThr^{180}/pTyr^{182}) ELISA Kit
For detection of phospho-p38 alpha (pThr^{180}/pTyr^{182}) in human or mouse cell and tissue lysates

Catalog Number **RAB0344**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The Phospho-p38 α (Thr^{180}/Tyr^{182}) ELISA (Enzyme-Linked Immunosorbent Assay) Kit is a very rapid, convenient, and sensitive assay kit that can monitor the activation or function of important biological pathways in human and mouse cell lysates. By determining phosphorylated p38 protein in the experimental model system, pathway activation can be verified in the cell lysates. One 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 and mouse phospho-p38 (Thr^{180}/Tyr^{182}). An antiphospho-p38 (Thr^{180}/Tyr^{182}) antibody has been coated onto a 96 well plate. Samples are pipetted into the wells and phosphorylated p38 (Thr^{180}/Tyr^{182}) present in a sample is bound to the wells by the immobilized antibody. The wells are washed and rabbit anti-p38 alpha MAPK antibody is used to detect phosphorylated p38. 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 p38 (Thr^{180}/Tyr^{182}) bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Components

1. Capture Antibody-Coated Microplate (Item A): 96 wells (12 strips × 8 wells) coated with antiphospho-p38 (Thr^{180}/Tyr^{182}).
2. 20× Wash Buffer Concentrate (Item B): 25 mL of 20× concentrated solution.
3. PhosphoELISA Lyophilized Positive Control Sample for Phosho-p38 (pThr^{180}) (Item K): 2 vials of lyophilized powder from treated Hela cell lysate.
4. Pan p38 Antibody (Item C2): 2 vials of rabbit anti-p38 alpha MAPK (1 vial is enough to assay half of a microplate).
5. HRP-conjugated Anti-Rabbit IgG Concentrate (Item D1): 1 vial (25 µL) of 500× concentrated HRP-conjugated anti-rabbit IgG.
6. TMB One-Step Substrate Reagent (Item H): 12 mL of 3,3′,5,5′-tetramethylbenzidine (TMB) in buffer solution.
7. Phosphorylation ELISA Stop Solution (Item I): 8 mL of 0.2 M sulfuric acid.
8. 5× Assay Diluent (Item E2): 15 mL of 5× concentrated buffer. For diluting cell lysate samples, detection antibody (Item C-2), and HRP-conjugated anti-rabbit IgG concentrate.
9. 2× Cell Lysate Buffer (Item J): 10 mL 2× cell lysis buffer (does not include protease and phosphatase inhibitors).

Reagents and Equipment Required but Not Provided.

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. Distilled or deionized water
8. Tubes to prepare sample dilutions

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

2× Cell Lysate Buffer should be diluted 2-fold with deionized or distilled water to yield 1× Cell Lysate Buffer (addition of protease and phosphatase inhibitors to 1× Cell Lysate Buffer is recommended prior to sample preparation).

Cell lysates - Rinse cells with PBS, making sure to remove any remaining PBS before adding the Cell Lysate Buffer. Solubilize cells at 4×10^7 cells/mL in 1× Cell Lysate Buffer. Pipette up and down to resuspend and 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, it is recommend to do serial dilution testing such as 5-fold and 50-fold dilution for the cell lysates with Assay Diluent (Item E2) 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.

Reagent Preparation

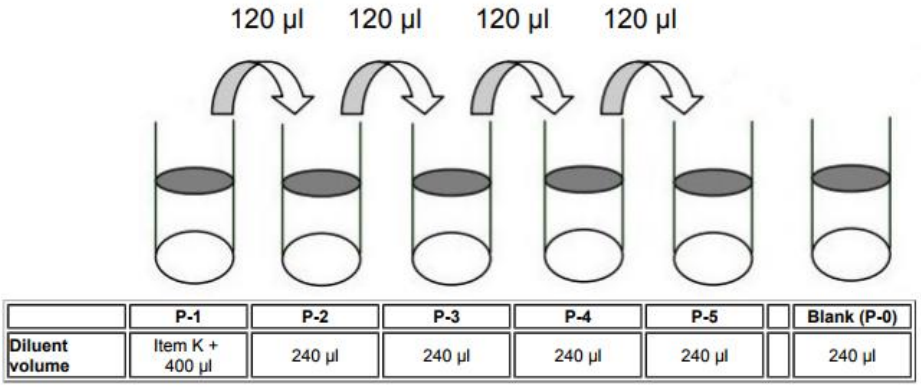
1. Bring all reagents and samples to room temperature (18–25 °C) before use.

2. 5× 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). The addition of protease and phosphatase inhibitors (not included) to the lysis buffer prior to use is recommended.

4. Preparation of Positive Control: Briefly spin the Positive Control Vial (Item K). Add 400 µL of prepared 1× 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 centrifugation of the positive control vial, and then pipetting the supernatant only for the assay. Pipette 240 µL of 1× Assay Diluent into each tube. Use the Positive Control (P-1) solution to produce a dilution series (see Figure 1). Mix each tube thoroughly before the next transfer. 1× Assay Diluent serves as the blank (P-0).

Figure 1.
Dilution Series for Positive Control



	P-1	P-2	P-3	P-4	P-5		Blank (P-0)
Diluent volume	Item K + 400 µl	240 µl	240 µl	240 µl	240 µl		240 µl

5. If the Wash Concentrate (20×) (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 1× Wash Buffer.

6. Preparation of rabbit anti-p38 alpha MAPK antibody: Briefly spin the vial of rabbit anti-p38 alpha MAPK (Item C-2). Add 100 µL of 1× 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 –70 °C for one month). The concentrate should then be diluted 55-fold with 1× Assay Diluent and used in Procedure, step 4.

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 1000-fold with 1× Assay Diluent and used in Procedure, step 6.
   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 10 mL 1× Assay Diluent to prepare a 1000-fold diluted HRP-conjugated anti-rabbit IgG solution.

Storage/Stability
Store the kit at –20 °C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles.

The reconstituted standard should be stored at –20 °C or –70 °C (–70 °C is recommended). Opened microplate strips or reagents may be store for up to 1 month at 2–8 °C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Procedure
1. Bring all reagents to room temperature (18–25 °C) before use. It is recommended that all samples and the Positive Control should be run at least in duplicate.

2. Add 100 µL of each sample or positive control into appropriate wells. Cover well with plate holder and incubate for 2.5 hours at room temperature or overnight at 4 °C with shaking.

3. Discard the solution and wash 4 times with 1× Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multichannel pipette or autowasher. 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.

4. Add 100 µL of prepared 1× rabbit anti-p38 alpha MAPK antibody (Preparation, step 6) to appropriate wells. Incubate for 1 hour at room temperature with shaking.

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

6. Add 100 µL of prepared 1× HRP-conjugated anti-rabbit IgG to corresponding well (Preparation, step 7). Incubate for 2 hours at room temperature with shaking.

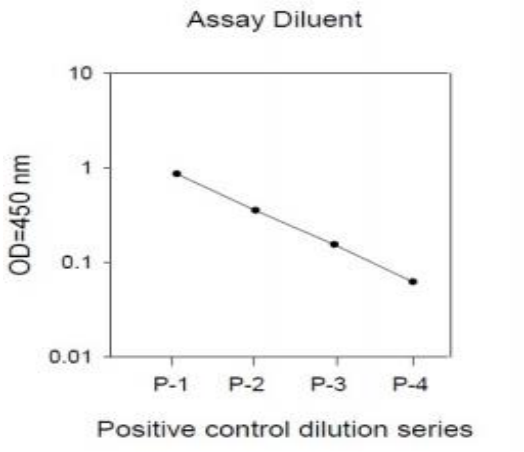
7. Discard the solution. Repeat the wash as in step 3.

8. 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 shaking.

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

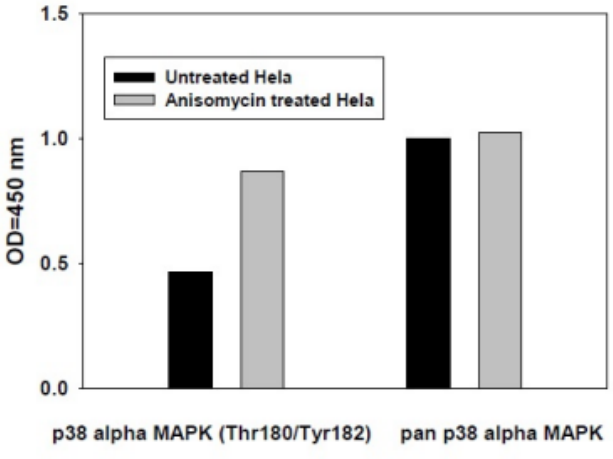
Results
Typical Data
ELISA data analysis: Average the duplicate readings for each sample or positive.

Positive Control:
Hela cells were treated with Anisomycin at 37 °C for 10 minutes. Cells were solubilzed at 4×10^7 cells/mL in Cell Lysate Buffer. Serial dilutions of lysates were analyzed with this ELISA (Please see Preparation, step 4 for detail).

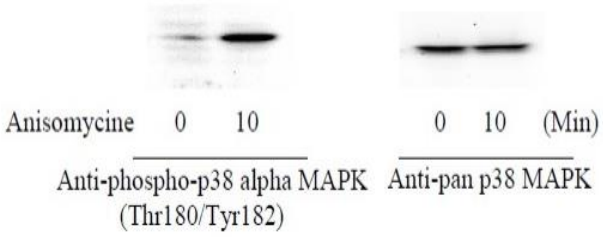


Recombinant Anisomycin Stimulation of Hela Cell Lines:
Hela cells were untreated or treated with Anisomycin for 10 minutes at 37 °C. Cell lysates were analyzed using this phospho ELISA and Western blot.

ELISA



Western blot



Appendix
Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 2 may change to over night
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at <–20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Stop solution should be added to each well before measurement.

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