User Guide

Phospho-p38 α (pThr^{180}/pTyr^{182}) ELISA Kit

RAB0344

Introduction

Phospho-P38 (Thr180/Tyr182) 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, Rat cell lysates. By determining phosphorylated p38 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, Mouse, Rat phospho-p38. An anti-pan p38 antibody has been coated onto a 96-well plate. Samples are pipetted into the wells and p38 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and rabbit anti-phospho-P38 (Thr180/Tyr182) 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 (Thr180/Tyr182) bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Storage/Stability

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 prepared reagent storage, see Kit Components below.

Components

- Anti-Pan-p38 Microplate: 96 wells coated with anti-pan-p38 antibody. Stable in storage for 1 month at −20 °C after preparation (Return unused wells to the pouch containing desiccant pack, reseal along entire edge).
- Positive Control: 1 vial of lyophilized powder from treated HeLa cell lysates. Stable in storage for 1 week at −80 °C after preparation.
- Anti-Phospho P38 (Thr180/Tyr182) Detection Antibody: 2 vials of rabbit anti-phospho-P38 (Thr180/Tyr182) (1 vial is enough to assay half of the microplate). Stable in storage for 5 days at 4 °C after preparation.
- HRP-conjugated antirabbit IgG: 1 vial (25 µL) of 500X concentrated HRP-conjugated anti-rabbit IgG. Do not store and reuse.
- Wash Buffer: 25 mL of 20X concentrated solution. Stable in storage for 1 month at 4 °C after preparation.
- Assay Diluent B: 15 mL of 5X concentrated assay diluent. Stable in storage for 1 month at 4 °C after preparation.
- Lysis Buffer: 5 mL of 2X cell lysis buffer. Stable in storage for 1 month at 4 °C after preparation.
- TMB One-Step Substrate Reagent: 12 mL of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution.
- Stop Solution: 8 mL of 0.2 M sulfuric acid.

Additional Materials Required (Not Provided)

- 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
- Absorbent paper
- Distilled or deionized water
- Log-log graph paper or computer and software for ELISA data analysis
- Tubes to prepare positive control or sample dilutions

Sample Preparation

Cell lysates - Rinse 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 Lysis Buffer (we recommend adding protease and phosphatase inhibitors to lysis buffer prior to sample preparation). 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, we recommend a serial dilution, such as a 5-fold to 50-fold dilution, for your cell lysates with prepared Assay Diluent (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.

Reagent Preparation

1. Bring all reagents and samples to room temperature (18–25 °C) before use.
2. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
3. Lysis Buffer 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. Add 400 µL of prepared 1X Assay Diluent into Positive Control. 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.
5. If the Wash Concentrate (20X) 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 rabbit anti-phospho-P38 (Thr180/Tyr182) antibody: Briefly spin the vial of rabbit anti-phospho-P38 (Thr180/Tyr182). 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 5 of the Assay Procedure.
7. Preparation of HRP-conjugated anti-rabbit IgG: Briefly spin the vial of HRP-conjugated anti-rabbit IgG concentrate before use. HRP-conjugated anti-rabbit IgG should be diluted vial (25 µL) of 500X with 1X Assay Diluent and used in step 7 of the Assay Procedure.

Assay Procedure

1. Bring all reagents and samples to room temperature (18–25 °C) before use. It is strongly recommended to run all positive controls and samples in at least duplicate.
2. 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 4 °C 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 auto washer. 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-P38 (Thr180/Tyr182) antibody (see Reagent Preparation step 6) into the well. 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 1 hour at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

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 4°C 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 1 hour at room temperature 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.

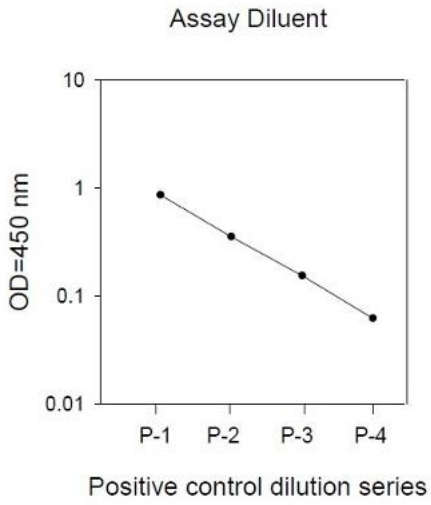
Calculation of Results

Typical Data

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

Positive Control

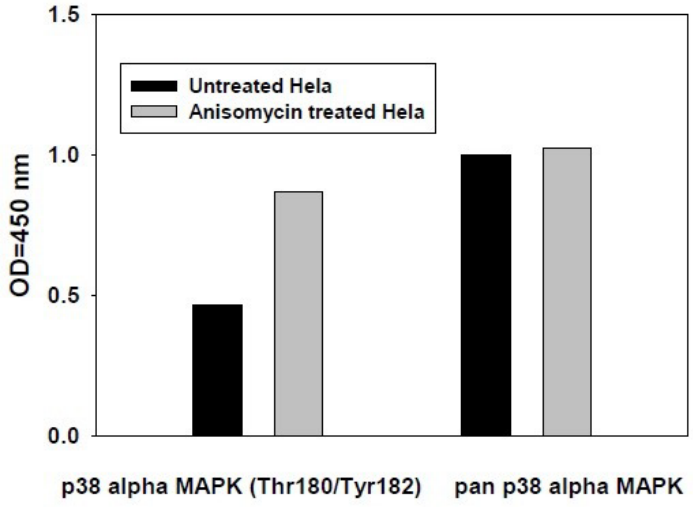
HeLa cells were treated with Anisomycin. Cells were solubilized at 4 x 10^7 cells/mL in Cell Lysate Buffer.



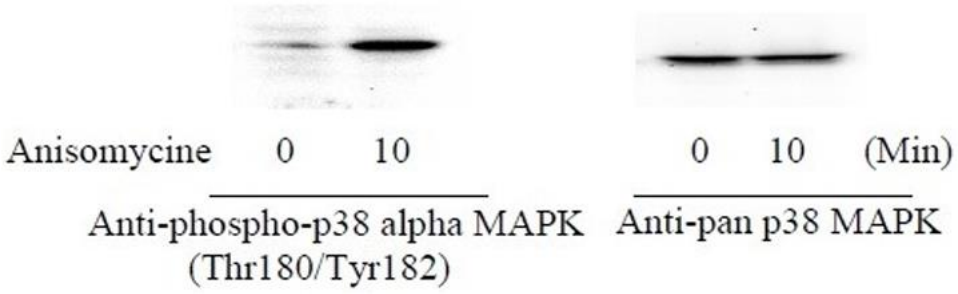
Anisomycin Stimulation of Hela Cell Lines

HeLa cells were treated or untreated with Anisomycin. Cell lysates were analysed using this phosphor ELISA and Western Blot.

ELISA



Western-Blot Analysis



Troubleshooting Guide

Problem	Cause	Solution
Low signal in samples	Sample concentration is too low	Increase sample concentration. Briefly spin down vials before opening. Dissolve the powder thoroughly.
	Improper preparation of detection antibody	
	Too brief incubation times	Ensure sufficient incubation time; Assay Procedure step 3 may be done overnight. Check pipettes and ensure correct preparation.
	Inadequate reagent volumes or improper dilution	
High signal in samples	Sample concentration is too high	Reduce sample concentration.
Large CV	Inaccurate pipetting. Air bubbles in wells.	Check pipettes. 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 positive control 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.
	Improper primary or secondary antibody dilution	Ensure correct dilution.

Notice

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RAB0344 Rev 02/26