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ab126445 - ERK1 (phospho T202 + Y204) + ERK2 (phospho T185 + Y187) + Total ERK1/2 ELISA Kit

Instructions for Use

For the quantitative measurement of Human, Mouse and Rat phosphorylated Erk1 (pT202/pY204) + Erk2 (pT185/pY187) + total Erk1/2 concentrations in cell lysates.

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

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Table of Contents

1. Introduction 2

2. Assay Summary 3

3. Kit Contents 4

4. Storage and Handling 5

5. Additional Materials Required 5

6. Preparation of Samples 6

7. Preparation of Reagents 7

8. Assay Method 9

9. Data Analysis 10

10. Troubleshooting 12

1. Introduction

ab126445 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 Erk1/2 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 phospho-Erk1 (T202/Y204)/Erk2 (T185/Y187) and total Erk1/2 in Human, Mouse and Rat cell lysates (help normalize the results of phospho-Erk1/2 from different cell lysate being compared). A total Erk1/2 antibody has been coated onto a 96-well plate. Samples are pipetted into the wells and Erk1/2 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and anti-phospho-Erk1 (T202/Y204)/Erk2 (T185/Y187) or anti-total-Erk1/2 is used to detect phosphorylated or total Erk1/2. 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 Erk1 (T202/Y204)/Erk2 (T185/Y187) or total Erk1/2 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

2. Assay Summary

Prepare all reagents, samples and standards as instructed.

⇩

Add 100 µl sample or positive control to each well. Incubate 2.5 hours at room temperature or overnight at 4°C.

⇩

Add 100 µl prepared primary antibody to each well. Incubate 1.0 hours at room temperature.

⇩

Add 100 µl prepared 1X HRP-conjugated secondary antibody solution to corresponding well. Incubate 1 hour at room temperature.

⇩

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

⇩

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

3. Kit Contents

- Erk1/2 Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-total Erk1/2 antibody.
- Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution.
- Assay Diluent (Item E): 15 ml of 5x concentrated buffer. For diluting cell lysate sample, detection antibody (Item C-1 and C-2) and secondary antibody (Item D-1) concentrate.
- Detection antibody Erk1 (T202/Y204)/Erk2 (T185/Y187) (Item C-1): 1 vial of rabbit anti-phospho-Erk1 (T202/Y204) /Erk2 (T185/Y187) (1 vial is enough to assay half microplate).
- Detection antibody Erk1/2 (Item C-2): 1 vial of rabbit anti-total-Erk1/2 (1 vial is enough to assay half microplate).
- HRP-conjugated anti-rabbit IgG (Item D-1), 25 µl of 500x HRP-conjugated anti-rabbit IgG concentrate.
- TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution.
- Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.

- Cell Lysis Buffer (Item J): 5 ml 2x cell lysis buffer (not including protease and phosphatase inhibitors).
- Positive Control (Item K): 1 vial of lyophilized powder from A431 cell lysate.

4. Storage and Handling

Upon receipt, the kit should be stored at –20°C. After initial use, Wash Buffer Concentrate (Item B), Assay Diluent (Item E), TMB One-Step Substrate Reagent (Item H), Stop Solution (Item I) and Cell Lysis Buffer (Item J) should be stored at 4°C to avoid repeated freeze-thaw cycles. Return unused wells to the pouch containing desiccant pack reseal along entire edge and store at –20°C. Item D-1 store at 2-8°C for up to one month (store at -20°C for up to 6 months, avoid repeated freeze-thaw cycles). Reconstituted Positive Control (Item K) should be stored at -80°C.

5. Additional Materials Required

- 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.

6. Preparation of Samples

Cell lysates - Rinse cells with PBS, making sure to remove any remaining PBS before adding the Cell Lysis Buffer. Solubilize cells at 4 x 107 cells/ml in 1x Cell Lysis Buffer (we recommend adding protease and phosphatase inhibitors to Cell 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 to do a serial dilution testing such as 5-fold and 50-fold dilution for your cell lysates with 1x Assay Diluent (Item E) 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.

Cell Lysis Buffer should be diluted 2-fold with deionized or distilled water before use (recommend to add protease and phosphatase inhibitors).

7. Preparation of Reagents

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Item E, Assay Diluent 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 250 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 buffer 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 Detection Antibodies:

a. Preparation of rabbit anti-phospho Erk1(T202/Y204)/Erk2(T185/Y187) antibody: Briefly spin the vial of rabbit anti-phospho Erk1(T202/Y204)/Erk2(T185/Y187). 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 6 of the Assay Procedure.

b. Preparation of rabbit anti-pan-Erk1/2 antibody: Briefly spin the vial of rabbit anti-pan-Erk1/2. 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 concentrate should then be diluted 55-fold with 1X Assay Diluent.

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 8 of the Assay Procedure.

8. Assay Method

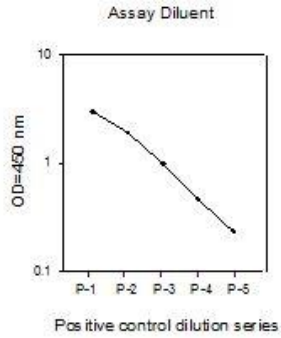
1. Bring all reagents to room temperature (18-25°C) before use. It is recommended that all samples or Positive Control should be run at least in duplicate.
2. Add 100 µl of each sample or positive control into appropriate wells. Cover well with plate holder and incubate for 2.5 hours at room temperature or overnight at 4°C with shaking.
3. Discard the solution and wash 4 times with 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 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 1x rabbit anti-phospho-Erk1 (T202/Y204)/Erk2 (T185/Y187) antibody or 1x rabbit anti-total-Erk1/2 (Preparation of Reagents step 5) to each well. Incubate for 1 hour at room temperature with shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µl of prepared 1x HRP-conjugated anti-rabbit IgG against rabbit anti-phospho-Erk1 (T202/Y204)/Erk2 (T185/Y187) antibody Incubate for 1 hour 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.

9. Data Analysis

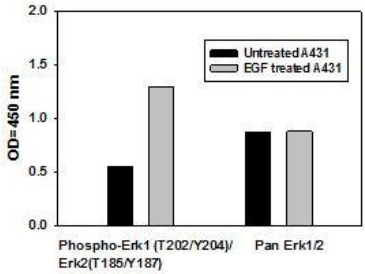
ELISA data analysis: Average the duplicate readings for each sample or positive.

i. **Positive Control:** A431 cells were treated with recombinant Human EGF at 37°C for 20 min. Solubilize cells at 4 x 107 cells/ml in Cell Lysis Buffer. Serial dilutions of lysates were analyzed in this ELISA. Please see step 3 of Preparation of Reagents for detail.

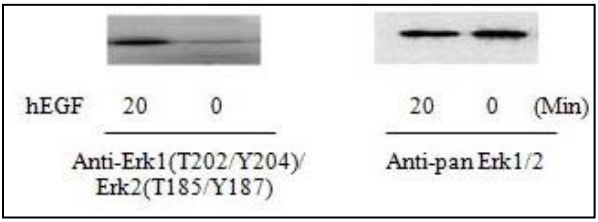


ii. **Recombinant Human EGF Stimulation of A431 Cell Lines**
A431 cells were treated or untreated with recombinant Human EGF for 10 min. Cell lysates were analyzed using this phosphoELISA and Western Blot.

a) ELISA



b) Western Blot Analysis



10. Troubleshooting

Problem	Cause	Solution
Sample signals	Too low: Sample concentration is too low.	Increasing sample concentration.
	Too high: Sample concentration is too high.	Reduce sample concentration.
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed.	Review the manual for proper washing. If using an automated plate washer, check that all ports are unobstructed.
	Contaminated wash buffer.	Make fresh wash buffer.
Positive Control: Low signal	Improper storage of the ELISA kit.	Upon receipt, the kit should be stored at -20°C. Store the positive control at -80°C after reconstitution.
Positive Control: Low signal	Stop solution	Stop solution should be added to each well before measurement and read OD immediately.
	Improper primary or secondary antibody dilution.	Ensure correct dilution.

Technical Support

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