Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) development ELISA Kit

Cat# E0002

Storage at 2-8 ℃ for 3 months

INTRODUCTION

This ELISA kit is used to measure phosphorylated human, mouse, and rat ERK1 (T202 / Y204) and ERK2 (T185 / Y187) in cell lysates.

SPECIFICATION

Detection range: 0.5 ng/mL ~ 30 ng/mL

Specificity: This kit is specifically recognizes ERK1 dually phosphorylated at T202 and Y204, and ERK2 dually phosphorylated at T185 and Y187.

PRINCIPLE of KIT

This ELISA kit contains the basic components required for the development of sandwich ELISAs to measure phosphorylated human, mouse, and rat ERK1 (T202 / Y204) and ERK2 (T185 / Y187) in cell lysates. An immobilized capture antibody specific for ERK1 / ERK2 binds both phosphorylated and unphosphorylated ERK1 / ERK2. After washing away unbound material, a biotinylated detection antibody is used to detect only phosphorylated receptor, utilizing a standard HRP format.

CONTENTS and STORAGE

Components	96T	Storage
Material		
Micro ELISA plate	96 wells * 2	2-8 ℃
Plate sealer	4 pieces	2-8 ℃
Sealed bags	2 bag	2-8 ℃
Instruction datasheet	1 copy	2-8 ℃
Reagents		
Human/Mouse/Rat Phospho-ERK1/ERK2 Capture Antibody	1 vial	Store for up to 1 month at 2-8 °C or aliquot and store at ≤ -20 °C or ≤ -70 °C for up to 3 months in a manual defrost freezer.
Human/Mouse/Rat Phospho-ERK1/ERK2 Detection Antibody	1 vial	
Human/Mouse/Rat Phospho-ERK1/ERK2 Standard	3 vial	Use within one hour of reconstitution. Use a fresh standard for each assay.
Streptavidin-HRP A	1 vial	Store for up to 3 months at 2-8 °C. DO NOT FREEZE.

Block buffer	90 mL	2-8 ℃
Stop Solution	15 mL	2-8 ℃
Substrate solution	30 mL	2-8 ℃
30X Wash Solution	60 mL	2-8 ℃

OTHER SUPPLIES REQUIRED

Pipettes and pipette tips, Microplate reader, Aprotinin, Leupeptin, Pepstatin, Phenylmethylsulfonyl Fluoride (PMSF), Sodium Fluoride (NaF), Triton X-100, Urea, Deionized or distilled water.

SOLUTIONS REQUIRED

PBS - 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.2-7.4, 0.2 µm filtered.

Diluent buffer #A - 1% BSA in PBS, pH 7.2-7.4, 0.2 µm filtered.

Diluent buffer #B - 1 mM EDTA, 0.5% Triton X-100, 5 mM NaF in PBS, pH 7.2-7.4.

Note: Diluent buffer #B is also the base buffer for Diluent buffer #C, Diluent buffer #D, and Lysis Buffer. Approximately 50 mL of this diluent is required to run the assay on one 96 well plate.

Diluent buffer #C - 1 mM EDTA, 0.5% Triton X-100, 5 mM NaF, 1 M Urea in PBS, pH 7.2-7.4.

Diluent buffer #D - 1 mM EDTA, 0.5% Triton X-100, 5 mM NaF, 6 M Urea in PBS, pH 7.2-7.4.

Lysis Buffer - 1 mM EDTA, 0.5% Triton X-100, 5 mM NaF, 6 M Urea, 10 µg/mL Leupeptin, 10 µg/mL Pepstatin, 100 µM PMSF, 3.0 µg/mL Aprotinin, 2.5 mM Sodium Pyrophosphate, 1 mM activated Sodium Orthovanadate in PBS, pH 7.2-7.4.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human/Mouse/Rat Phospho-ERK1/ERK2 Capture Antibody - Each vial contains 1440 µg/mL of mouse anti-human ERK1/ERK2 antibody when reconstituted with 200 µL of PBS.

Human/Mouse/Rat Phospho-ERK1/ERK2 Detection Antibody - Each vial contains 14.4 µg/mL of biotinylated rabbit anti-human phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) antibody when reconstituted with 1.0 mL of Diluent buffer #A. Immediately before use, dilute the detection antibody to a working concentration of 400 ng/mL in Diluent buffer #A. Prepare only as much detection antibody as required to run each assay.

Human/Mouse/Rat Phospho-ERK1/ERK2 Standard - Refer to the vial label for the stock concentration of recombinant human phospho-ERK2 (T185/Y187) when reconstituted with 500 µL of Diluent buffer #D. An initial 6-fold dilution should be made in Diluent buffer #B. Further dilutions should be made in Diluent buffer #C immediately before use. A seven point curve using 2-fold serial dilutions and a high standard of 30 ng/mL is recommended.

Streptavidin-HRP A - 1.0 mL of Streptavidin conjugated to horseradishperoxidase. Immediately before use, dilute the Streptavidin-HRP A to the working concentration specified on the vial label using Diluent buffer #A.

PREPARATION of SAMPLE

Cell Lysates - Rinse cells two times with PBS, making sure to remove any remaining PBS after the second rinse. Solubilize cells at 1 x 10^7 cells/mL in Lysis Buffer and allow samples to sit on ice for 15 minutes. Assay immediately or store at ≤ -70

°C. Before use, centrifuge samples at 2000 x g for 5 minutes and transfer the supernate to a clean test tube. Sample protein concentration may be quantified using a total protein assay. For assaying, dilute lysates 6-fold with Diluent buffer #B and make further serial dilutions in Diluent buffer #C.

PRECAUTIONS

- The Stop Solution recommended for use with this kit is an acid solution.
- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- Color Reagent B recommended for use with this kit may cause skin, eye, and respiratory irritation. Avoid breathing fumes.
- Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

TECHNICAL HINTS AND LIMITATIONS

- This ELISA should not be used beyond the expiration date on the kit label.
- Individual results may vary due to differences in technique, plasticware, and water sources.
- It is important that the diluents selected for reconstitution and for dilution of the samples and standard reflect the environment of the samples being measured. The diluents suggested in this protocol should be suitable for most cell lysates.
- The type of enzyme and substrate and the concentrations of capture/detection antibodies used can be varied to create an immunoassay with a different sensitivity and dynamic range. A basic understanding of immunoassay development is required for the successful use of these reagents in immunoassays.
- A thorough and consistent wash technique is essential for proper assay performance.
- Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Use a fresh reagent reservoir and pipette tips for each step.
- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay. Buffers containing protein should be made under aseptic conditions and stored at 2-8 °C or be prepared fresh daily.

PROTOCOL

Plate Preparation

1. Dilute the capture antibody to a working concentration of 8.0 µg/mL in PBS, without carrier protein. Immediately coat a 96 well microplate with 100 µL per well of the diluted capture antibody. Seal the plate and incubate overnight at room temperature.

2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of 3 washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, 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 by inverting the plate and blotting it against clean paper towels.

3. Block plates by adding 300 µL of Block Buffer to each well. Incubate at room temperature for 1-2 hours.

4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Prepare Standard Working Solution

Standard 7	30 ng/mL	Standard (30 ng/mL)
Standard 6	15 ng/mL	150 µl Standard 7 + 150 µl Diluent buffer #C
Standard 5	7.5 ng/mL	150 µl Standard 6 + 150 µl Diluent buffer #C
Standard 4	3.75 ng/mL	150 µl Standard 5 + 150 µl Diluent buffer #C
Standard 3	1.875 ng/mL	150 µl Standard 4 + 150 µl Diluent buffer #C
Standard 2	0.9375 ng/mL	150 µl Standard 3 + 150 µl Diluent buffer #C
Standard 1	0.46875 ng/mL	150 µl Standard 2 + 150 µl Diluent buffer #C
Blank	0 ng/L	Diluent buffer #C

2. Add 100 µL of sample or standard in Diluent buffer #C per well. Use Diluent buffer #C as the zero standard. Cover with a plate sealer and incubate 2 hours at room temperature.
3. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
4. Add 100 µL of the diluted detection antibody to each well. Cover with a new plate sealer and incubate 2 hours at room temperature.
5. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
6. Add 100 µL of the diluted Streptavidin-HRP A to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
7. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
8. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
9. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Calculate of result

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density (O.D.). Results may be normalized to total protein or cell number.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human/mouse/rat phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

PRODUCT USE LIMITATION

These products are intended for research use only.