ab284557 – ERK1 (phospho T202 + Y204) + ERK2 (phospho T185 + Y187) Translocation Assay Kit (Cell-Based)

For the detection of nuclear translocation of phospho-ERK1 and phospho-ERK2 in mammalian cells.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284557

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Fixative Solution	10 mL	-20°C
Blocking Buffer	10 mL	-20°C
Wash Buffer	75 mL	-20°C
Phospho-ERK1/2 Primary Antibody (100X)	50 µL	-20°C
Secondary Antibody (100X)	50 µL	-20°C
Tamoxifen (1000X)	30 µL	-20°C
DAPI (1000X)	20 µL	-20°C

Materials Required, Not Supplied

Tissue culture plates and appropriate culturing media
Phosphate Buffered Saline (PBS) (pH 7.4)
0.1% Gelatin Solution (optional, only required for suspension cells)
Fluorescence microscope (570 nm excitation and UV filter)

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Fixative Solution, Blocking Buffer, Wash Buffer: Ready to use. After opening, store at 4°C and protected from light.

Primary and Secondary Antibodies (100X): After opening, aliquot and store at –20°C in the dark. Keep on ice while in use. Avoid freeze and thaw cycle.

Tamoxifen and DAPI: Store at –20°C after opening. Completely thaw before each use. Avoid multiple freeze and thaw cycles.

Assay Protocol

This assay was developed with MCF-7 cells and can be modified for any suspension or adherent cell line. The protocol below refers to a 96-well tissue culture plate format and the assay volume is 100 µL. Adjust the volumes accordingly for other plate formats. Cell number per well and assay conditions should be optimized based on cell line specifications. Cells should be grown, treated, fixed and stained directly in multi-well plates. Bring all buffers to room temperature prior to the experiment. All steps should be carried out at room temperature unless otherwise specified.

Preparation of control and experimental wells

1. Subculture cells of interest in appropriate medium to desired confluency.
2. The day before the experiment, seed a 96-well plate with 1 X10^4 viable cells in 100 µL volume per well and incubate overnight at 37°C, 5% CO_2 to allow cell attachment.
3. For suspension cells: add 125 µL of 0.1% gelatin solution into each well, tilt the plate to cover the entire well surface and place it in a tissue culture hood for 1 hour. Gently remove the 0.1% gelatin solution and seed your cells with 100 µL medium.
4. Your experiment should always consist of parallel negative, positive and experimental wells respectively.
5. The next day, apply desired treatments to the experimental wells omitting the negative and positive control wells.
6. To use Tamoxifen as positive control, dilute Tamoxifen (1000X) directly into the culture medium of positive control wells to obtain 1:1,000 - 1:10,000 dilution.
7. Incubate the plate for the period of time required by your experimental protocol.
8. Upon completion, gently aspirate off the culture medium from all wells and rinse cells briefly with 200 µL of 1X PBS.
9. For suspension cells: centrifuge the plate at 200 g (or the lowest centrifuge setting) for 3 minutes to gently deposit the cells onto the surface. Tilt the plate and gently remove the media by aspirating with a pipette tip.
10. Rinse cells briefly with 200 µL of PBS and spin again.
11. It is important to avoid excessive centrifugation speeds, which can damage the cells.
12. Make note of the place that is used and perform subsequent aspirations from the same place.

Permeabilization and Blocking

1. Remove PBS and incubate the cells with 100 µL of Fixative Solution for 15 min in the dark.
2. Remove Fixative Solution by gentle aspiration for adherent cells or centrifuge the plate at 200 g for 3 minutes followed by gentle aspiration for suspension cells.
3. Wash cells three times with 100 µL of Wash Buffer 5 min each.
4. Remove the Wash Buffer.
5. For suspension cells: centrifuge the plate between each wash at 200 g for 3 minutes.
6. Incubate cells with 100 µL of Blocking Buffer for 30 minutes.
7. Remove the Blocking buffer by aspiration after centrifugation at 200 g for 3 minutes.
8. While blocking, prepare the primary antibody and proceed to Immunofluorescence Staining.

Immunofluorescence Staining

Δ Note: *The recommended dilution for primary and secondary antibodies is 1:100 but it may vary for different cell lines. To prevent cells from drying and photobleaching, the plate should be always covered and protected from light during the incubation periods.*

1. Primary Antibody Incubation: Dilute the Phospho-ERK1/2 Primary Antibody 1:100 in Wash Buffer.
2. Add 100 µL of antibody dilution into each well.
3. Incubate the plate for 2 hours at room temperature, or for best results overnight at 4°C.
4. Remove the antibody by aspiration or centrifugation for suspension cells.
5. Rinse cells briefly three times with 100 µL of Wash Buffer and remove the washes.
6. Secondary Antibody Incubation: Dilute the Secondary Antibody 1:100 in Wash Buffer.
7. Add 100 µL of antibody dilution into each well.
8. Incubate the plate for 2 hours at room temperature in the dark, or overnight at 4°C fridge protected from light.

9. Repeat the removal of antibody and wash from step 3a.
10. DAPI Staining: Dilute DAPI stain 1:1000 in Wash Buffer, aliquot 100 µL to each well and incubate for 10 minutes in the dark.
11. Remove the stain and rinse wells with 100 µL of Wash Buffer.
12. For removal of DAPI stain and wash follow the steps from primary antibody incubation.
13. Add 100 µL of PBS into each well.
14. Cells are ready to be imaged.
15. For later analysis, store the plate at 4°C in the dark.
16. Examine the staining under fluorescence microscope with 570 nm excitation and UV laser for Phospho-ERK1/2 Secondary Antibody and DAPI respectively.

Technical Support

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