ab284564 – Phospho-p38 MAPK (Thr180+Tyr182) Translocation Assay Kit (Cell-Based)

For the visualization of translocation of activated p38 MAPK 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/ab284564

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
Blocking Buffer	10 mL	-20°C
Fixative Solution	10 mL	-20°C
DAPI (1000X)	10 µl	-20°C
P38 Secondary Antibody (100X)	100 µl	-20°C
Phospho-p38 MAPK Primary Antibody (100X)	100 µl	-20°C
Thrombin	50 µl	-20°C
Wash Buffer	2 x 75 mL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 24 or 48 or 96-well clear bottom tissue culture plate and appropriate culturing media
- Phosphate Buffered Saline (PBS) (pH 7.4)
- Fluorescence microscope (550 nm excitation and UV filter for DAPI)

Reagent Preparation

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

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

Phospho-p38 Primary Antibody (100X), p38 Secondary Antibody (100X): Aliquot and store at –20°C in dark after opening. Keep on ice while in use.

Thrombin: Aliquot and store at –20°C after opening. Completely thaw before each use. Avoid freeze and thaw cycle.

Assay Protocol

Δ Note: The protocol below refers to a 96-well tissue culture plate format; the assay volume is 100 µl and it should be adjusted accordingly for other plate formats. Cell number per well should be optimized based on cell line specifications. Assay conditions optimization is strongly recommended. Bring all buffers to room temperature prior to the experiment. Cells should be grown, treated, fixed and stained directly in multi-well plates. All steps should be carried out at room temperature unless otherwise specified.

1. Preparation of control and experimental wells:

a) Subculture cells of interest in appropriate medium until desired confluency. The day before the experiment, seed a 96-well plate with 3 x 10^4 viable cells per well in a 100 µl volume and incubate overnight at 37°C, 5% CO_2. Your experiment should always consist of parallel negative, positive and experimental wells respectively.

b) The next day, apply the desired treatment to the experimental wells omitting the negative control wells. Dilute Thrombin (10-50 times) directly into the positive control wells to induce the nuclear translocation of phosphorylated p-38. Incubate the plate for 24 hours, or for the period of time required by your experimental protocol.

c) Terminate the experiment by removing culture medium from all wells. Rinse cells briefly with 200 µl PBS and proceed to the Permeabilization and Blocking protocol.

2. Permeabilization and Blocking:

a) Remove PBS and incubate the cells with 100 µl Fixative Solution for 10 min.

b) Remove Fixative Solution and wash cells three times with 100 µl Wash Buffer (5 min each). Gently remove the Wash Buffer.

c) Incubate cells with 100 µl Blocking Buffer for 60 min. While blocking, prepare the primary antibody and proceed to Immunofluorescence Staining Protocol.

3. Immunofluorescence Staining:

Δ Notes: *The recommended dilution for primary and secondary antibodies is 1:100 but it may vary for different cell lines. During the incubation the plate should always be covered and protected from light to prevent drying and photobleaching.*

a) **Primary Antibody Incubation:** Dilute the Phospho-p38 MAPK Primary Antibody 1:100 in Wash Buffer. Gently remove Blocking Buffer and apply 100 µl diluted Primary Antibody into each well. Incubate the plate for 1 hour at room temperature (for best results, incubate the plate overnight at 4°C). Wash cells three times with 200 µl Wash Buffer for 5 min each. Proceed to the incubation with the secondary antibody.

b) **Secondary Antibody Incubation:** Dilute the p38 Secondary Antibody 1:100 in Wash Buffer, apply 100 µl into each well and incubate for 1 hour at room temperature, or overnight at 4°C. Upon completion, wash cells two times with 200 µl Wash Buffer for 5 min each.

c) **DAPI Staining:** Dilute DAPI stain 1:1000 in Wash Buffer, add 100 µl to each well and incubate for 10 minutes. Remove the stain and wash all wells with Wash Buffer one time for 5 minutes. Add 100 µl of PBS into each well. Cells are ready to be imaged. Store the plate at 4°C in the dark for later analysis

d) Examine the staining under fluorescence microscope with 550 nm excitation laser for (p38) and UV laser (for DAPI).

Technical Support

For all technical or commercial enquiries please go to:

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www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)