Phospho-p38 MAPK (Thr180+Tyr182) Translocation Assay Kit (Cell-Based) (#BN01125)

(Catalog # BN01125; 100 assays; Store at -20°C)

I. Introduction:

Mitogen-activated protein kinases (MAPKs) are proline-directed serine and threonine protein kinases that regulate numerous physiological and pathophysiological cell responses. p38 MAPKs belong to a class that plays an important role during inflammatory processes, and their expression is upregulated by inflammatory and stress stimuli (cytokines, ultraviolet irradiation, osmotic and heat shock). p38 MAPKs are also involved in autophagy, apoptosis, cell differentiation, and are implicated in the regulation of inflammatory mediators levels such as tumor necrosis factor-α (TNF-α) and cyclooxygenase-2 (COX-2). Accumulating evidence suggests that p38 MAPKs play an important role in arthritis, and inflammation of the liver, kidney, brain, and lung. Diseases induced by chronic inflammation, including gastritis, colitis, dermatitis, rheumatoid arthritis, pulmonary diseases and type II diabetes affect millions of people every year. Therefore, p38 is an important target in better understanding of inflammation and development of novel treatments. p38 MAPK is activated following phosphorylation at Thr180/Tyr182 by upstream MKK6 and MKK3 kinases. While in quiescent cells p38 localizes to both nucleus and cytosol, stress induced conformational changes within the active site result in selective translocation of activated-p38 into the nucleus. Thus, the selective nuclear accumulation of p38 could be a mechanism to facilitate the phosphorylation of p38 MAPK nuclear targets. Assay Genie's Phospho-p38 MAPK (Thr180+Tyr182) Translocation Assay Kit (Cell-Based) provides a valuable tool to detect the nuclear translocation of activated p38 in fixed mammalian cells.

II. Applications:

- Detection of nuclear translocation of phosphorylated-p38 in mammalian cells
- Screening effectors of p38 MAPK signaling pathway
- Detection of cellular stress, DNA damage, heat shock, and pro-inflammatory stimuli

III. Sample Type:

- Adherent or suspension mammalian cell cultures

IV. Kit Contents:

Components	BN01125	Cap Code	Part Number
Fixative Solution	10 ml	WM	BN01125-1
Blocking Buffer	10 ml	NM	BN01125-2
Wash Buffer	2 X 75 ml	NM	BN01125-3
Phospho-p38 MAPK Primary Antibody (100X)	100 µl	Brown	BN01125-4
p38 Secondary Antibody (100X)	100 µl	Red	BN01125-5
Thrombin	50 µl	Yellow	BN01125-6
DAPI (1000X)	10 µl	Blue	BN01125-7

V. User Supplied Reagents and Equipment:

- A 96-, 48, or 24-well clear tissue culture plate and appropriate culturing media
- Phosphate Buffered Saline (PBS) (pH 7.4)
- Fluorescence microscope capable of measuring EX/EM at 550 nm and equipped with UV filter for DAPI

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **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.

VII. p38 Nuclear Translocation Assay Protocol:

Notes:

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 Protocol:

Notes:
The plate should be kept in dark 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 phospho-p38 staining under a fluorescent microscope with 550 nm excitation laser and UV laser (for DAPI).

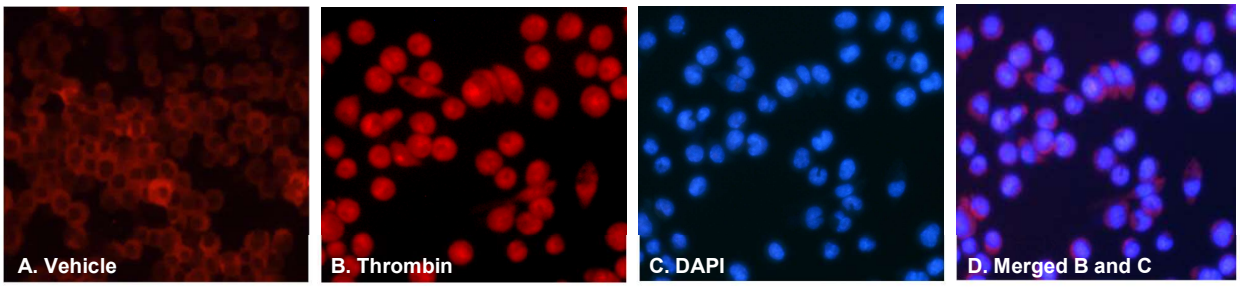

Figure: Thrombin-induced activation and translocation of Phopho-p38 MAPK (Thr180/Tyr182) in J774 macrophages. J774 cells were seeded into a 96-well plate at 3 $X10^4$ cells/well and cultured overnight followed by a 24 hr treatment with either vehicle (A) or Thrombin diluted 1:100 directly into the well (B). Subsequently, cells were processed according to the provided protocol. Translocation of phosphorylated-p38 from cytoplasm to nuclei upon stimulation by Thrombin is apparent with DAPI staining (C) and merged images (D).

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