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Product Information

ANTI-p38 MAP Kinase
produced in rabbit, IgG fraction of antiserum

Catalog Number **M0800**

Product Description

Anti-p38 MAP Kinase is produced in rabbit using a synthetic peptide K-DEVISFVPPPLDQEEMES derived from the C-terminus of mouse p38 MAP kinase (amino acids 343-360 with N-terminally added lysine) as immunogen. This sequence is highly conserved in human and rat p38 MAP kinase. The peptide is coupled to KLH. Whole antiserum is purified to provide an IgG fraction of antiserum.

Anti-p38 MAP Kinase reacts in immunoblotting with p38 MAP kinase using NIH 3T3 mouse fibroblasts cell lysate and rat brain extract. Staining of the 40 kDa band is specifically inhibited with the immunizing peptide.

Mitogen-activated protein kinases (MAP kinases, MAPKs) are family of protein kinases which are considered to play a crucial role in signal transduction pathways in mammalian cells by leading mitogenic signals to their intracellular targets.[1,2] MAP kinases regulate several cellular processes, such as proliferation, differentiation, response to stress and oncogenesis. Several MAP kinase subgroups have been identified in mammalian cells among them are the extracellular signal-regulated kinases (ERKs) isoforms[1,3] (including ERK1 and ERK2), the c-Jun N-terminal kinases isoforms (JNK1, JNK2 also termed SAPKs)[4] and the p38 MAP kinase (p38 MAPK also termed RK, Hog1 and CSBP).[1,5-8] Each of these subgroups seems to operate in separate MAP kinase signaling cascades. p38 MAPK is activated in response to stress signals including osmolarity changes, heat shock, UV-irradiation, cytokines, and lipopolysaccharides (LPS).[5-7,9] The activation pathway involves the MAP kinase kinases, MKK3, MKK4 and MKK6.[10,11] p38 MAP kinase contains a dual phosphorylation sequence Thr-Gly-Tyr (TGY) that is required for full activation. This phosphorylation motif differs from the Thr-Glu-Tyr (TEY) sequence found in ERK1 and ERK2 and the Thr-Pro-Tyr (TPY) sequence found in JNK, but is identical in Hog1, a p38 MAPK homolog of the yeast *Saccharomyces cerevisiae*.[5,7] p38 MAP kinase phosphorylates MAPK-activated protein kinase 2 (MAPKAPK-2), which in turn phosphorylates the heat shock proteins hsp 25/27[6,12] and the transcription factors ATF2 and CHOP.[13,14] p38 MAP kinase appears to be widely expressed, in the brain, muscle, thymus, spleen, heart, lung, kidney, and is expressed in high levels in PC12 cells and in fibroblasts. Antibodies that react specifically with p38 MAP kinase are useful for the study of the specific activation requirements, differential tissue expression, intracellular localization of MAP kinase isoforms in normal and neoplastic tissue.

Anti-p38 MAP Kinase may be used for the detection of p38 MAP kinase by immunoblotting using cell culture extracts/lysates, and brain tissue.

Reagent

Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as preservative.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C. For continuous use, the product may be stored at 2-8 °C for up to one month. For extended storage, freeze in working aliquots at –20 °C. Repeated freezing and thawing, or storage in “frost-free” freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure

Reagents and Equipment for Sample Preparation

1. Rats (Sprague-Dawley).
2. NIH 3T3 mouse fibroblasts culture.
3. Phenylmethylsulfonyl fluoride (PMSF), Catalog Number P7626, 0.5 M in EtOH.
4. Pepstatin A , Catalog Number P4265, 2 mg/ml in DMSO.
5. Leupeptin, Catalog Number L2884.
6. Aprotinin, Catalog Number A4529.
7. DTT, Catalog Number D9760.
8. Homogenization Buffer (Rat brain), Ice Cold:
   20 mM Tris.HCl pH 7.5 containing
   0.25 M sucrose, 10 mM EGTA, 2 mM EDTA, and protease inhibitors: 1 mM PMSF, 50 μg/ml leupeptin, 25 μg/ml aprotinin, 10 μg/ml pepstatin A, and 2 mM dithiothreitol (DTT).

Note: Add all protease inhibitors freshly before tissue homogenization.

9. Lysis Buffer (NIH 3T3 cells), Ice Cold:
   40 mM Hepes pH 7.5 containing 1% Triton® X-100, 5 mM EGTA, 1mM dithiothreitol, 1mM sodium vanadate, 1 mM benzamidine, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 2 μg/ml pepstatin
10. Homogenizer: Mechanical homogenizer.
11. Refrigerated High-Speed Centrifuge.
12. Protein assay kit .
13. Laemmli sample (3x) buffer containing 2-mercaptoethanol.

Preparation of Rat Brain Extract

Note: All procedure steps are carried out at 0-4 °C, unless noted otherwise.

1. Rapidly remove whole brains (5 g) from Sprague-Dawley rats (4-5 rats, 250-300 g) and collect into ice cold homogenization buffer.
2. Homogenize tissue in 5x volumes of ice cold homogenization buffer (w/v) using mechanical homogenizer at maximum speed (3 x 10 sec pulses with 1 minute rest in between).
3. Centrifuge homogenate at 1200 x *g* for 10 minutes at 4 °C. Collect supernatant.
4. Centrifuge supernatant at 15,000 x *g* for 20 minutes at 4 °C. Collect clear supernatant.
5. Remove 0.5 ml aliquot of supernatant for protein determination using the Bradford method with BSA as standard.
6. Add to supernatant 3x sample buffer to final dilution of 1x sample buffer.
7. Boil samples for 5 minutes at 95 °C.
8. Aliquot samples of rat brain extract and store at –70 °C.

Preparation of NIH3T3 Cell Lysate

1. Grow cells to confluence in 10 cm plate containing 10% FCS in DMEM.
2. Remove medium from culture dish.
3. Rinse plates with ice cold PBS (2 x 10 ml).
4. Add 0.7 ml/plate of freshly prepared lysis buffer and scrape cells.
5. Remove cell lysate to a microcentrifuge tube and leave for 30 minutes on ice.
6. Centrifuge lysate at 12,000 x *g* for 20 min at 4 °C.
7. Collect supernatant containing cytosolic fraction.
8. Remove 0.2 ml aliquot of lysate for protein determination by the Bradford method using BSA as standard.
9. Add to supernatant 3x sample buffer to final dilution of 1x sample buffer.
10. Boil sample for 5 minutes at 95 °C.
11. Aliquot 0.1 ml sample of fibroblast cells extract and store at –70 °C.

Reagents and Equipment for Immunoblotting

1. Rat brain extract or NIH 3T3 fibroblasts lysate.
2. 10% polyacrylamide slab minigel with 5% stacking gel (80 x 80 x 1.5 mm).
3. Nitrocellulose membrane (0.45 mm). Prestained HMW markers, Catalog Number C3312.
4. Blocking Buffer : 10% dry milk (w/v) in 10 mM phosphate buffered saline (PBS), pH 7.4.
5. Dilution Buffer: 1% BSA in PBS pH 7.4 containing 0.05% TWEEN® 20.
6. Washing Buffer: PBS pH 7.4 containing 0.05% TWEEN 20.
7. p38 MAP Kinase peptide (amino acids 343-360 with N-terminally added lysine). Dissolve in deionized, distilled water at 0.5 mg/ml. Store aliquots at –20 °C.
8. Primary antibody: Anti-p38 MAP Kinase at appropriate dilution in dilution buffer.
9. Secondary Antibody: Anti-Rabbit IgG- Alkaline Phosphatase, Catalog Number A9919, at appropriate dilution in dilution buffer.
10. Substrate: BCIP/NBT Tablets, Catalog Number B5655.
11. Electrophoresis and transfer apparatus.

Immunoblotting Procedure

Note: In order to obtain best results in different preparations it is recommended to optimize procedure conditions (antibody dilutions, incubation times, blocking conditions etc.), for a specific application.

1. Resolve rat brain extract (100 mg) on precast 10% polyacrylamide minigel, or NIH 3T3 cells lysate (0.3 ml) on precast 10% polyacrylamide minigel (1x 10 cm confluent dish/4 minigels).

2. Run SDS-PAGE at 20mA/gel at room temperature.
3. Perform transfer (36mA) to nitrocellulose membrane for 1 hour at room temperature.
4. Block nitrocellulose membrane in blocking buffer for at least 1 hour at room temperature.
5. Incubate membrane with primary antibody dilutions for 2 hours at room temperature.[(a)]
6. Wash membrane with washing buffer 4 x 5 minutes.
7. Incubate membrane with secondary antibody at recommended dilution in dilution buffer for 1 hour at room temperature.
8. Wash membrane with washing buffer 4 x 5 minutes Wash 1 x 5 minutes in deionized water.
9. Dissolve ALP substrate tablet each in 10 ml deionized water. Incubate membrane with substrate solution.
10. Wash membrane thoroughly with deionized water.
11. Air-dry blots on filter paper.(a)Note: For specific inhibition of p38 MAP kinase band (40 kDa band) it is recommended to incubate prediluted antibody with p38 MAP kinase peptide (concentration 1-5 µg/ml), for 2 hours at room temperature or overnight at 4 °C.

Product Profile

Immunoblotting, a minimum working antibody dilution of 1:10,000 is determined using rat brain extract and mouse NIH 3T3 fibroblasts cell lysate.

Note: In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

References

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