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ProductInformation

Anti-MAP Kinase Phosphatase-1 (MKP-1)
Antibody developed in Rabbit
IgG Fraction of Antiserum

Product Number **M 3787**

Product Description

Anti-MAP Kinase Phosphatase-1 (MKP-1) is developed in rabbit using a synthetic peptide DERSASLDGAKRD derived from the N-terminus of mouse MAP kinase phosphatase-1 (amino acids 87-99), conjugated to KLH as immunogen. This sequence is highly conserved in human MKP-1 homolog (CL-100), and is different in other MAP kinase phosphatases (MKP-2, PAC-1, B23). Whole antiserum is fractionated and then further purified by ion-exchange chromotography to provide the IgG fraction of antiserum which is essentially free of other rabbit serum proteins.

By Immunoblotting (SDS-PAGE), Anti-MAP Kinase Phosphatase-1 (MKP-1) is specific for MAP kinase phosphatase-1 (MKP-1, 39 kDa protein) using TPA-induced A431 cells extract. Staining of the 39 kDa band is specifically inhibited with the MAP kinase phosphatase-1 peptide (mouse, amino acids 87-99).

Mitogen-activated protein kinases (MAP kinases, MAPKs) are a family of serine/threonine protein kinases, which are considered to play a crucial role in signal transduction pathways in mammalian cells leading mitogenic signals and stress signals to their intracellular targets.[1,2] MAP kinases regulate several cellular processes including proliferation and differentiation. They may also play a role in oncogenesis. Several MAP kinase subgroups have been identified in mammalian cells including the well characterized ERK1 and ERK2 (p42 and p44 MAPK). Activation of MAP kinases involves specific phosphorylation on threonine and tyrosine residues within the Thr-Xaa-Tyr motif by dual specificity protein threonine/tyrosine kinase, MAP kinase kinases (MEK). Phosphorylation of both residues is required for MAP kinase activation. The inactivation of MAP kinase is a critical event that regulates the physiological response to MAP kinase activation. This inactivation seems to involve, in part, the immediate action of non-specific protein serine/threonine phosphatases such as PP2A, and in the later stages by dual specificity MAP kinase-specific phosphatases called MAP kinase phosphatases (MKPs).[3-6] MKPs dephosphorylate both threonine and tyrosine residues of activated MAP kinase.[5] Thus, the activation of MAP kinase appears to be tightly regulated through the coordinated action of MEK and MKPs. The MAP kinase phosphatases comprise an expanding family of protein threonine/tyrosine phosphatases[4-6,9] with sequence similarity to a serine/tyrosine phosphatase encoded by the late H1 gene of vaccinia virus.[3] These include murine MKP-1 (also called 3CH134 or Erp), human PAC-1, CL-100 and B23, and yeast Yop51, MSG5. Recently a novel MKP, MKP-2, has been cloned from PC-12 cells.[9] All are dual specificity phosphatases which are expressed in a wide range of cells and tissues and are capable of dephosphorylating MAP kinase *in vitro* and *in vivo*. The human CL-100 gene is rapidly induced by oxidative stress and heat shock in human skin fibroblasts. The murine homolog MKP-1 (3CH134) is an immediate early gene that is rapidly and transiently expressed during the G_0/G_1 transition and is induced by mitogenic stimulation including serum, PDGF, FGF or TPA. Its rapid transcription (induced within minutes) and subsequent translation, following growth factor stimulation, have been suggested to provide an additional control over the mitogenic signalling pathway in which an immediate early gene product feeds back to modulate growth factor signals. Overexpression of MKP-1 was shown to dramatically inhibit fibroblast proliferation, suggesting that inactivation of MAP kinase by MKP-1 *in vivo* has a profound negative effect on cellular proliferation.[10] Antibodies that react specifically with MAP kinase phosphatase isoforms are useful for the study of the specific activation requirements, differential tissue expression, and for intracellular localization of MAP kinase phosphatases in normal and neoplastic tissue.

Reagents

The product is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer
Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability
For continuous use, store at 2-8 °C for a maximum of one month. For extended storage freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure
Immunoblotting Procedure of A431 Whole Cell Extract

Reagents and Equipment
1. A431 cell culture.
2. Phorbol 12-myristate 13-acetate (O-Tetradecanoyl-phorbol 13-acetate, TPA), (Product No. P 8139), stock solution 1mg/ml in DMSO. Store at 20 °C.
3. Laemmli SDS-PAGE sample buffer (2x) containing 2-mercaptoethanol.

Preparation of TPA-Induced A431 Whole Cell Extract
1. Grow cells to confluence in 10cm plate containing 10% FCS in DMEM.
2. Add TPA to cell culture to final concentration of 50 ng/ml.
3. Incubate cell culture for 1 hour at 37 °C.
4. Remove medium from culture dish.
5. Rinse plates with PBS (2 x 10 ml).
6. Scrape cells and add 0.5 ml/plate of Laemmli SDS-PAGE sample buffer (2x).
7. Boil sample for 5 min. at 95 °C.
8. Centrifuge extract at 12,000 x *g* for 5 min at 4 °C.
9. Aliquot sample of A431 whole cells extract and store at –70 °C.

Immunoblotting Reagents and Equipment
1. TPA-induced A431 whole cell extract.
2. 10% polyacrylamide slab minigel with 5% stacking gel (80 x 80 x 1.5mm).
3. Nitrocellulose membrane (0.45 µm).
4. Prestained LMW markers (Product No. C 3312).
5. Blocking Buffer: 10% dry milk (w/v) in 10 mM phosphate buffered saline (PBS), pH 7.4.
6. Dilution Buffer: 1% BSA in PBS pH 7.4 containing 0.05% Tween 20.
7. Wash Buffer: PBS pH 7.4 containing 0.05% Tween 20 (Product No. P 2194).
8. MAP kinase phosphatase-1 (MKP-1) peptide (mouse, amino acids 87-99). Dissolve in double distilled water at 0.5mg/ml. Store aliquots at –20 °C.
9. Primary antibody: IgG fraction Anti-MAP Kinase Phosphatase-1 (rb) at appropriate dilution.
10. Secondary Antibody: Alkaline phosphatase Anti-rabbit IgG (Gt) (Product No. A 9919) at appropriate dilution in dilution buffer.
11. Substrate: BCIP/NBT (Product No. B 5655).
12. 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 whole cells extract of TPA-induced A431 (50 ml/well) on precast 10% polyacrylamide minigel.
2. Run SDS-PAGE at 20mA/gel at room temp.
3. Perform transfer (36mA) for 1 hour at room temperature to nitrocellulose membrane.
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 times for 5 minutes each.
7. Incubate membrane with secondary antibody at recommended dilution for 1 hour at room temp.
8. Wash membrane with washing buffer 4 times for 5 minutes each. Wash once for 5 minutes in deionized water.
9. Dissolve each BCIP\NBT substrate tablet in 10ml 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 MAP kinase phosphatase-1 band (39 kD) it is recommended to incubate prediluted antibody with MAP kinase phosphatase-1 peptide 5-20 mg/ml, (final concentration), for 2 hours at room temperature or overnight at 4 °C.

Product Profile
A minimum working dilution of 1:5,000 is determined by immuno-blotting using a TPA-induced A431 whole cell extract.

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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