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ProductInformation

ANTI-MAP KINASE-ACTIVATED PROTEIN KINASE-2 (MAPKAPK-2)
Developed in Rabbit, IgG Fraction of Antiserum

Product Number **M 3550**

Product Description

Anti-MAP Kinase-Activated Protein Kinase-2 (MAPKAPK-2) is developed in rabbit using a synthetic peptide KEDKERWEDVKEEMTS derived from the C-terminus of human MAPKAPK-2 (amino acids 343-358), conjugated to KLH as immuongen. This sequence is identical in mouse MAPKAPK-2. Whole antiserum is fractionated and then further purified by ion-exchange chromatography to provide the IgG fraction of antiserum that is essentially free of other rabbit serum proteins.

Anti-MAP Kinase-Activated Protein Kinase-2 (MAPKAPK-2) specifically recognizes human MAPKAPK-2 (45 kDa protein) by immunoblotting. Staining of the 45 kDa band is specifically inhibited with the MAPKAPK-2 immunizing peptide.

MAP kinase activated protein kinase-2 (MAPKAP kinase-2, MAPKAPK-2) is a serine/threonine protein kinase[1] that is activated by the p38 MAP kinase in response to various signals including mitogenic and stress stimuli. [2,3,4] MAPKAPK-2 is distinguished from the RSK family of ribosomal protein S6 kinases (also termed MAPKAPK-1), which are activated by the MAP kinases ERK1 and ERK2, based on its substrate specificity and amino acid sequence.[1,5] Upon activation p38 MAP kinase activates the downstream MAPKAPK-2 by phosphorylation of the threonine residue in the Pro-Thr motif in response to heat shock or mitogenic stimuli. Activated MAPKAPK-2, in turn, phosphorylates the small heat shock protein hsp25/hsp27, both *in vitro* and *in vivo,* at serine residues. Thus, hsp25/hsp27 seems to be the physiological substrate of MAPKAPK-2.[6,7]

The catalytic domain of MAPKAPK-2 shows similarity (35-40% identity) to several calmodulin-dependent protein kinases and the C-terminal domain of MAPKAPK-1. It is preceded by a proline rich domain in the N-terminal region that may interact with SH3 domain containing proteins.[5,8] Two isoforms of MAPKAPK-2 (45 and 50 kDa) have been identified that may be generated by alternative splicing of a common precursor mRNA. They differ in their C-terminals, the longer form containing a putative nuclear localization signal $KK(X)_{10}KRRKK$. The MAPKAPK-2 mRNA transcript appears to be widely distributed in mammalian tissues.[5,9] In contrast, MAPKAPK-2 from different species have been reported to have characteristic molecular masses (45 – 60 kDa range).[1,3,4] Antibodies that react specifically with MAPKAPK-2 are useful for the study of the differential tissue expression, intracellular localization of MAPKAPK-2 in normal and neoplastic tissue.

Reagent

Anti-MAP Kinase-Activated Protein Kinase-2 (MAPKAPK-2) is supplied as an IgG fraction of anti-serum 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 sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution.
Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2 °C to 8 °C for up to 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. Working dilution samples should be discarded if not used within 12 hours.

Procedure

<u>Immunoblotting procedure for MDBK whole cell extract</u>

A. Reagents and Equipment:

- MDBK cell culture.
- Laemmli SDS-PAGE sample buffer (1x) containing 2-mercaptoethanol.
- 10% polyacrylamide slab minigel with 5% stacking gel (80 x 80 x 1.5 mm).
- Nitrocellulose membrane (0.45 μm).

- Prestained LMW markers (Sigma C3312).
- Blocking Buffer : 10% dry milk (w/v) in 10 mM phosphate buffered saline (PBS), pH 7.4.
- Dilution Buffer: 1% BSA in PBS pH 7.4 containing 0.05% Tween-20.
- Washing Buffer: PBS pH 7.4 containing 0.05% Tween-20.
- MAP kinase-activated protein kinase-2 (MAPKAPK-2) (human, amino acids 343 358). Dissolve in dionized water at 0.5 mg/ml. Store aliquots at –20 °C.
- Primary antibody: Anti-MAP Kinase-Activated Protein Kinase-2 (MAPKAPK-2) (M 3550) at appropriate dilution in dilution buffer.
- Secondary Antibody: Anti-rabbit IgG - Alkaline phosphatase conjugate (A 9919) at appropriate dilution in dilution buffer.
- Substrate: BCIP/NBT Tablets (B 5655).
- Electrophoresis and transfer apparatus.

B. Preparation of MDBK whole cell extract

1. Grow cells to confluence in 10cm plate containing 10% FCS in DMEM.
2. Remove medium from culture dish.
3. Rinse plates with PBS (2 x 10 ml).
4. Scrape cells and add 2.0 ml/plate of Laemmli SDS-PAGE sample buffer (1x).
5. Boil sample for 5 min. at 95 °C.
6. Centrifuge extract at 12,000 x g for 5 min at 4 °C.
7. Aliquot sample of MDBK whole cells extract and store at –70 °C.

C. Immunoblotting

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 MDBK whole cells extract (200 µl/slab) on precast 10% polyacrylamide minigel.
2. Run SDS-PAGE at 20 mA/gel at room temperature.
3. Perform transfer (36 mA) 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, 5 min. each.
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 times, 5 min. each. Wash once, for 5 min., in deionized water.
9. Dissolve BCIP/NBT 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 MAPKAPK-2 band (45 kDa) it is recommended to incubate prediluted antibody with MAPKAPK-2 peptide 5.0-10 µg/ml (final concentration) for 2 hours at room temperature or overnight at 4 °C.

Product Profile

A minimum working dilution of 1:2,000 is determined by immunoblotting using using MDBK whole cell extract.

Note: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References

1. Stokoe, D., et al., EMBO J. **11** 3985-3994 (1992).
2. Rouse, J., et al., Cell **78** 1027-1037 (1994).
3. Cuenda, A., et al., FEBS Lett. **364** 229-233 (1995).
4. Cano, E., et al., Oncogene **12** 805-812 (1996).
5. Stokoe, D., et al., Biochem. J. **296** 843-849 (1993).
6. Stokoe, D., et al., FEBS Lett. **313** 307-313 (1992).
7. Zhou, M., et al., J. Biol. Chem. **268** 35-43 (1993).
8. Engel, K., et al., FEBS Lett. **336** 143-147 (1993).
9. Zu, Y.-L., et al., Biochem. Biophys. Res. Commun. **200** 1118-1124 (1994).

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