ProductInformation

ANTI-MAP KINASE ACTIVATED PROTEIN KINASE-3 (MAPKAPK-3)
Developed in Sheep, Affinity Isolated Antibody

Product Number **M7681**

Product Description

Anti-MAP Kinase Activated Protein Kinase-3 (MAPKAPK-3) is developed in sheep using a synthetic peptide K-QAGSSSASQGCNNQ[G] that corresponds to the amino acids 368-382 of human MAPKAPK-3 conjugated to KLH/BSA as immunogen. Affinity isolated antibody is obtained by peptide immunoaffinity chromatography.

Anti-MAP Kinase Activated Protein Kinase-3 (MAPKAPK-3) reacts specifically with human MAPKAPK-3 (42 kD) by immunoprecipitation. The antibody does not react with MAPKAPK-2. The antibody does not interfere with kinase activity.

Anti-MAP Kinase Activated Protein Kinase-3 may be used for immunoprecipitation of active MAPKAPK-3 in cell lysates but is not recommended for immunoblotting.

The mitogen-activated protein kinase (MAPK) signal transduction cascade is characterized by a series of kinases, each of which subsequently phosphorylates and activates the next kinase in the cascade. For example, MAPK-activated protein kinase (MAPKAPK) is a serine/threonine kinase that is phosphorlyated and activated by the MAP kinase homologue, stress-activated protein kinase-2 (SAPK-2). SAPK is activated in response to some form of cellular stress and can initiate adaptive responses, such as apoptosis. Following activation, MAPKAPK in turn phosphorylates substrates with the sequence H-X-R-X-X-S where H is any hydrophobic residue[1]. Three different MAPKAPK's have been identified to date. MAPKAPK-1 differs in response to inhibitors and amino acid sequence[2]. MAPKAPK-2 and -3 are both activated in response to cellular stress, interleukin-1 and tumor necrosis factor in KB and HeLa cells[1,3]. Their amino acid sequence shows 75% homology to each other and both have similar structural features including a proline-rich N-terminus, a nuclear localization signal and conservation of key amino acid residues (Thr-222, Ser-272 and Thr-334)[3,4]. It is thought that MAPKAPK-2 and –3 are likely to have either overlapping or identical substrates *in vivo*[1]. Both MAPKAPK-2 and MAPKAPK-3 activity can be inhibited with SB 203580 (Sigma Product No. S8307).

Reagents

The product is supplied as the affinity isolated antibody in 35mM Tris-glycine, pH 7.4, containing 30% glycerol.

Protein concentration is approximately 0.36 mg/ml by Bradford analysis.

Storage/Stability

Store at 0°C to –20°C. Aliquot to avoid repeated freezing and thawing. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure

1. Add 10 µg of Anti-MAPKAP Kinase 3 to a microcentrifuge tube.
2. Add 100 µl of a 1:1 (v/v) Protein G-Agarose (Sigma Product No. P2294) slurry that has been washed with ice-cold PBS.
3. Add 200 µl of ice-cold PBS.
4. Incubate for 30 minutes to 1 hour at RT or overnight at 4°C in ice-cold PBS, rotating the sample so the antibody and protein G mix thoroughly.
5. Pellet at 14,000 rpm for 15 seconds.
6. Remove the supernatant and then wash the Protein G-agarose twice with ice-cold Buffer A (50 mM Tris, pH 7.5, 1 mM EDTA, 1 mM EGTA, 0.5 mM Na_3VO_4, 0.1% 2-mercaptoethanol, 1% Triton X-100, 5 mM sodium pyrophosphate, 10 mM sodium glycero-phosphate, 0.1 mM PMSF, 1 µg/ml aprotinin, 1 µg/ml leupeptin and 50 mM NaF). Note: Add PMSF fresh just before using buffer.
7. Resuspend the pellet of washed beads in 100 µl of ice-cold Buffer A.
8. Add sample containing antigen to beads.[a]
9. Add 200 µl of ice-cold PBS
10. Incubate for 2 hours at 4°C, rotating the sample to mix thoroughly.

11. Wash the antigen/antibody/protein G-agarose complex with 500 µl of ice-cold Buffer A containing 0.5 M NaCl. Repeat the wash.
12. After the second wash, remove the supernatant and resuspend the pellet in 20 to 50 µl of Laemmli sample buffer (2% SDS, 10% glycerol, 0.05 M Tris, pH 6.8, containing bromphenol blue as the dye marker). Heat for 5 minutes at 100°C.
13. Microcentrifuge for 5 seconds. Transfer the supernatant to a fresh tube.
14. If a non-reducing gel is to be run, the sample can be loaded directly. If a reducing gel is to be run, add 5% 2-mercaptoethanol, incubate 1 hour 37°C then load sample onto gel.
15. After running the gel, analyze by protein staining, immunoblotting, or autoradiography (if the sample was radiolabeled).

[a] In order to obtain the best results, we recommend trying several amounts of sample to a given amount of beads in order to determine the optimal condition for immunoprecipitation.

Product Profile
Recommended use: 5 µg of Anti-MAP Kinase Activated Protein Kinase-3 will immunoprecipitate active MAPKAPK-3 from a cell lysate of HeLa cells stimulated with 0.5 mM sodium meta-arsenite.

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

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3. McLaughlin, M.M., Kumar, S., McDonnell, P.C., Van Horn, S., Lee, J.C., Livi, G.P. and Young, P.R., J. Biol. Chem., **271**, 8488 (1996).
4. Sithanandam, G., Latif, F., Duh, F.M., Bernal, R., Smola, U., Li, H., Kuzmin, I., Wixler, V., Geil, L. and Shrestha, S., Molec. Cell. Biol., **16**, 868 (1996).

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