



3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

Product Information

Anti-MAGI-2

Developed in Rabbit
IgG Fraction of Antiserum

Product Number **M 2441**

Product Description

Anti-MAGI-2 is developed in rabbit using a synthetic peptide corresponding to amino acids 554-571 of MAGI-2/S-SCAM conjugated to KLH as immunogen. The sequence is conserved in rat, human, and mouse MAGI-2/S-SCAM. 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-MAGI-2 specifically recognizes rat MAGI-2/S-SCAM by immunoblotting (approx. 180, 160, and 105 kDa) and does not cross react with other members of the family. Staining of MAGI-2/S-SCAM in immunoblotting is specifically inhibited by the immunizing peptide.

The MAGUK (Membrane-Associated Guanylate Kinase) family of proteins is characterized by the presence of multi-PDZ and SH3 domains, and a single region of homology to *Saccharomyces cerevisiae* guanylate kinase (GuK) domain. Its prototypic member is PSD95.¹ All MAGUKs studied to date localize to regions of cell-cell contact, such as tight junctions in epithelial cells and synaptic junctions in neurons, and are believed to be involved in the assembly of multiprotein complexes via their protein-protein interaction domains.² Using different screening assays, three novel closely related MAGUK proteins were isolated: MAGI-1/BAP1, MAGI-2/S-SCAM/ARIP, and SLIPR/MAGI-3. Similar to other MAGUK family members, MAGI-1, -2, and -3 are characterized by the presence of multi-PDZ domains, and a guanylate kinase domain. Its distinct feature is WW domains that replace the characteristic SH3 domains present in other MAGUK proteins. Different components interacting with these proteins in a complex were identified.³⁻⁸

MAGI-2 was first identified in rat, as a protein interacting with N-methyl-D-aspartate receptors and neuronal cell adhesion proteins, and was named S-SCAM.^{5,9} Three isoforms of S-SCAM were identified, S-SCAM α , β , and γ , of 1277, 1113, and 1053 amino acids length, respectively.¹⁰ MAGI-2 as well as MAGI-3, was shown to interact with the tumor suppressor PTEN through one of its PDZ domains, apparently acting as a scaffolding protein that could assemble a multi subunit signaling

complex.⁷ Further evidence of their scaffolding role, is the isolation of MAGI-2 as a binding partner of the β_1 -adrenergic receptor, as well as of β -catenin, providing a link between the two proteins.^{11,12}

Antibodies reacting specifically with MAGI-2/S-SCAM may be useful in studying the expression and function of the protein, as well as for distinguishing between MAGI-1, -2, and -3.

Reagent

Anti-MAGI-2 is supplied 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 hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at -20 °C. Repeated freezing and thawing is not recommended. Storage in frost-free freezers is also not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

A minimum working dilution of 1:250 is determined by immunoblotting using a rat brain extracts.

Note: In order to obtain the best results using different techniques and preparations, we recommend determining the optimal working dilutions by titration.

Procedure

For immunoblotting of rat brain extracts, we strongly recommend the addition of 5% non-fat dry milk in the

blocking and antibody dilution solutions, as indicated in the following protocol.

All incubation steps should be performed at room temperature.

1. Separate proteins from rat brain extracts using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol.
2. Transfer proteins from the gel to nitrocellulose membrane.
3. Block the membrane using a solution of Dulbecco's phosphate buffered saline containing 5% non-fat dry milk (DPBS, Product No. D 8537; non-fat dry milk, Product No. M 7409) for at least 60 minutes.
4. Wash the membrane three times for 10 minutes each in PBS containing 0.05% Tween 20 (Product No. P 3563).
5. Incubate the membrane with an optimized concentration of Anti-MAGI-2, diluted in PBS containing 0.05% Tween 20 and 5% non-fat dry milk for at least 60 minutes.
6. Wash the membrane three times for 10 minutes each in PBS containing 0.05% Tween 20.
7. Incubate the membrane with anti-rabbit IgG peroxidase conjugate (Product No. A 0545) as the secondary antibody, at the recommended concentration, in PBS containing 0.05% Tween 20 and 5% non-fat dry milk. Incubate for 60 minutes at room temperature.

8. Treat the membrane with a peroxidase substrate.

References

1. Anderson, J.M., Curr. Biol., **6**, 382-384 (1996).
2. Fanning, A.S., and Anderson, J.M., Curr. Biol., **6**, 1385-1388 (1996).
3. Dobrosotskaya, I., et al., J. Biol. Chem., **272**, 31589-31597 (1997).
4. Shiratsuchi, T., et al., Biochem. Biophys. Res. Commun., **247**, 597-604 (1998).
5. Hirao, K. et al., J. Biol. Chem., **273**, 21105-21110 (1998).
6. Shoji, H., et al., J. Biol. Chem., **275**, 5485-5492 (2000).
7. Wu, X., et al., Proc. Natl. Acad. Sci. USA, **97**, 4233-4238 (2000).
8. Wu, Y., et al., J. Biol. Chem., **275**, 21477-21485 (2000).
9. Wood, J.D., et al., Mol. Cell. Neuroscience, **11**, 149-160 (1998).
10. Hirao, K., et al., J. Biol. Chem., **275**, 2966-2972 (2000).
11. Xu, J., et al., J. Biol. Chem., **276**, 41310-41317 (2001).
12. Nishimura, W., et al., J. Neuroscience, **22**, 757-765 (2002).

KAA/NV 08/02