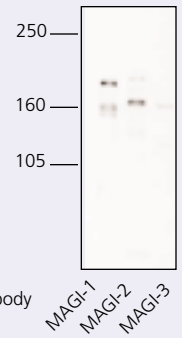
New Product Highlights

Antibodies to MAGI-1, MAGI-2, Slipr/MAGI-3: Assemblers of multiprotein complexes

The MAGUK (membrane associated guanylate kinase) family of proteins, whose prototypic member is PS95, is characterized by the presence of multi-PDZ and SH3 domains, and a single region of homology to the *Saccharomyces cerevisiae* guanylate kinase (Guk) domain [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 systems, three novel closely related MAGUK proteins were isolated: MAGI-1/BAP1, MAGI-2/rSCAM and Slipr/MAGI-3. Similar to other MAGUK members, these three proteins have the characteristic multi-PDZ domains and a guanylate kinase domain. However, a WW domain replaces the characteristic SH3 domain present in other MAGUK proteins. The different components interacting in a complex with these proteins were identified. MAGI-1 was first identified in mouse as a protein interacting with K-RasB, a GTP-binding protein that initiates the MAP kinase cascade [3]. Three isoforms were identified and named MAGI-1a, MAGI-1b and MAGI-1c, comprised of 1139, 1171 and 1374 amino acid residues, respectively. MAGI-2 was initially identified in rat, as a protein interacting with N-methyl-D-aspartate (NMDA) glutamate receptors and neuronal cell adhesion proteins, and was named S-SCAM [4]. Three isoforms of S-SCAM were identified, comprised of 1277, 1113 and 1053 amino acid residues, respectively [5]. MAGI-3 (also termed Slipr) was identified by yeast two hybrid screening as a protein interacting with the tumor suppressor PTEN [6] and receptor tyrosine phosphatase β (RPTPβ) [7]. The overall homology among the MAGI proteins, especially regarding the structural domains, is high.

Immunoblotting with Anti-MAGI-1, Anti-MAGI-2/S-SCAM and Anti-Slipr/MAGI-3



Rat brain extracts were separated by SDS-PAGE and blotted with **Anti-Rabbit MAGI-1** (Prod. No. **M 5691**), **Anti-MAGI-2** (Prod. No. **M 2441**) and **Anti-Slipr/MAGI-3** (Prod. No. **S 1190**). The antibodies were visualized with Goat Anti Rabbit IgG, Peroxidase Conjugate (Prod. No. **A 0545**) using a chemiluminescent substrate.

Specific Recognition of MAGI Isoforms by Anti-MAGI Antibodies

Anti-MAGI-1 **Anti-MAGI-2/ S-SCAM**

Rat brain extracts were separated by SDS-PAGE and blotted with Anti-MAGI-1 (Prod. No. **M 5691**) or Anti-MAGI-2 (Prod. No. **M 2441**).
Lane 1: Anti-MAGI Antibody
Lane 2: Anti-MAGI Antibody + 10 mg/ml MAGI-1 peptide
Lane 3: Anti-MAGI Antibody + 10 mg/ml MAGI-2 peptide

The antibodies were visualized with Goat Anti-rabbit IgG, Peroxidase Conjugate (Prod. No. **A 0545**) using a chemiluminescent substrate.

Anti-Slipr/MAGI-3

Extracts of 293-T cells transfected with rat Slipr were separated by SDS-PAGE, and blotted with:
Lane 1: Anti-Slipr/MAGI-3 (Prod. No. **S 1190**)
Lane 2: Anti-MAGI-1 (Prod. No. **M 5691**)
Lane 3: Anti-MAGI-2 (Prod. No. **M 2441**)

The antibodies were visualized with Goat Anti-rabbit IgG, Peroxidase Conjugate (Prod. No. **A 0545**) using a chemiluminescent substrate.

Sigma-RBI has developed antibodies that specifically recognize these MAGUK proteins. **Anti-MAGI-1** (Prod. No. **M 5691**), **Anti-MAGI-2** (Prod. No. **M 2441**) and **Anti-Slipr/MAGI-3** (Prod. No. **S 1190**) are developed using synthetic peptides. **Monoclonal Anti-Slipr/MAGI-3** (Prod. No. **S 4191**) is also available. Anti-MAGI-1 recognizes human and mouse MAGI-1 (approx. 170, 130 and 120 kDa), Anti-MAGI-2 recognizes human, mouse and rat MAGI-2 (approx. 180, 160 and 105 kDa) and Anti-Slipr/MAGI-3 recognizes rat Slipr/MAGI-3 (approximately 140-160 kDa). The antibodies are carefully tested by immunoblotting and are able to distinguish between the three proteins.

References

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3. Dobrosostakaya, I., et al., *J. Biol. Chem.*, **272**, 31589-31597 (1997).
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5. Hirao, K. et al., *J. Biol. Chem.*, **275**, 2966-2972 (2000).
6. Wu, Y., et al., *J. Biol. Chem.*, **275**, 21477-21485 (2000).
7. Adamsky, K. et al., *J. Cell Sci.*, In press (2003).