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ProductInformation

ANTI-l-AFADIN
Developed in Rabbit
Affinity Isolated Antibody

Product Number **A 0349**

Product Description

Anti-l-Afadin is developed in rabbit using as immunogen a synthetic peptide corresponding to amino acid residues 1814-1829 of rat afadin with N-terminal cysteine conjugated to maleimide-activated keyhole limpet hemocyanin (KLH). The sequence of the peptide is identical in several human AF-6 isoforms. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-l-Afadin specifically recognizes l-afadin by immunoblotting (~200 kDa). Additional weak bands may be detected in some extract preparations. Staining of the l-afadin band is specifically inhibited with the immunizing peptide. The antibody is also useful for the detection of l-afadin by immunocytochemistry and immunohistochemistry. The antibody reacts with l-afadin of human, dog, rat, and mouse.

Afadin is a cell-cell adherens junction F-actin binding multidomain protein. Two splice variants of afadin have been described: l-afadin, which is ubiquitously expressed, and the smaller s-afadin, which is abundantly expressed in neural tissue. l-Afadin contains one PDZ domain near its middle followed by three proline-rich and one F-actin-binding region at the carboxyl-terminal. s-Afadin is an approximate 190 kDa protein that lacks both the third proline-rich and the F-actin binding regions. It is homologous to the human AF-6 (ALL-1 fusion partner from chromosome 6), gene product. The AF-6 gene product is fused to the ALL-1 gene in a subset of human acute myeloid leukemias [translocation involving 11q23, t(6;11)(q27;q23)].[1]

l/s-Afadin is localized to cell-cell junctions and binds several cytoplasmic proteins such as the small GTPase Ras, the tight junction protein ZO-1,[2] and the vinculin binding protein ponsin/SH3P12.[3] In addition, l-afadin also binds through its PDZ several integral membrane components such as the Ca^{2+}-independent homophilic immunoglobulin-like nectin, the Junctional Adhesion Molecule (JAM),[4] and a subset of the EphB receptor protein-tyrosine kinases.[5] AF-6/Afadin contains two potential N-terminal Ras-binding protein domains, the first of which has been shown to interact with Ras and Rap1A GTPases.[6] Profilin, a key regulator of actin polymerization has also been shown to interact with AF-6.[6] AF-6/Afadin appears to play a major role in the generation and in the proper organization of adherens junctions and tight junctions. Absence of the AF-6 homologue in mice results in early embryonic lethality , most likely due to cell-cell junction disorganization and defects in the embryonic ectoderm polarity.[7]

Reagent

Anti-l-Afadin is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.

Antibody concentration: 0.9-1.5 mg/ml

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

For immunoblotting, a minimum working antibody dilution of 1:2,000 is recommended using an extract of rat brain.

For indirect immunofluorescent staining, a minimum working antibody dilution of 1:500 is recommended using cultured dog MDCK kidney cells and cultured human HepG2 cells.

For indirect immunofluorescent staining of frozen mouse liver sections, a minimum working antibody dilution of 1:1,000 is recommended.

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

References

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5. Buchert, M., et al., J. Cell Biol., **144**, 361-371 (1999).
6. Boettner, B., et al., Proc. Natl. Acad. Sci. USA, **97**, 9064-9069 (2000).
7. Ikeda, W., et al., J. Cell Biol., **146**, 1117-1131 (1999).

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