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Product Information

Anti-Early Endosomal Antigen 1 (N-terminal)

produced in rabbit, affinity isolated antibody

Catalog Number **E4156**

Synonym: Anti- EEA1

Product Description

Anti-Early Endosomal Antigen 1 (N-terminal) is produced in rabbit using a synthetic peptide corresponding to amino acid residues 24-40 of human EEA1 with C-terminal added cysteine, conjugated to KLH, as immunogen. The corresponding sequence is identical in mouse. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-Early Endosomal Antigen 1 (N-terminal) recognizes human, mouse, and rat EEA1. Applications include immunoblotting (~160 kDa) and immuno-fluorescence. Detection of the EEA1 band by immunoblotting is specifically inhibited by the immunizing peptide.

Early Endosomal Antigen 1, a 162 kDa autoantigen associated with subacute systemic lupus erythematosus that specifically localized to early endosomes, is a regulator of endocytic membrane docking and fusion.^{1,2} EEA1 is a dimer,³ which comprises extensive coiled-coil regions. At its C-terminus, it contains a cysteine-rich zinc-finger-like domain named FYVE domain that is implicated in the specific localization of EEA1 to endosomes. This FYVE domain is conserved from yeast to man among several proteins involved in intracellular trafficking.¹ The FYVE zinc-finger domain binds specifically to the membrane lipid phosphatidylinositol 3-phosphate (PtdIns(3)P) in a Zn²⁺-dependent manner. Anchoring of the FYVE domain to PtdIns(3)P-enriched membranes is pH-dependent due to a pair of conserved histidine residues, being enhanced by the acidic cytosolic environment.⁴ Endosomal targeting of EEA1 also requires its binding to the active form of the small GTPase Rab5. The binding of EEA1 to PtdIns(3)P and Rab5-GTP is essential for the localization and function of EEA1 in endocytic membrane fusion.^{2, 5-6}

Anti-EEA1 may be used as an early endosome marker.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody concentration: ~1 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 extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is 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

Immunoblotting: a working concentration of 0.4-0.8 µg/mL is recommended using a whole extract of mouse NIH-3T3 cells applying a chemiluminescent detection reagent.

Indirect immunofluorescence: a working concentration of 5-10 µg/mL is recommended using human HeLa and rat NRK cells.

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

References

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3. Callaghan, J., et al., *Biochem. J.*, **338**, 539-543 (1999).
4. Lee, S.A., et al., *Proc. Natl. Acad. Sci. USA*, **102**, 13052-13057 (2005).
5. Simonsen, A., et al., *Nature*, **394**, 494-498 (1998).
6. Gillooly, D.J., et al., *Biochem. J.*, **355**, 249-258 (2001).

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