

EEA1 siRNA (m): sc-35264

BACKGROUND

Early endosomes are cytoplasmic compartments that function in receiving and sorting endocytosed proteins for vesicular transport. EEA1 (early endosome antigen 1) is a peripheral membrane protein that co-localizes with the transferrin receptor and Rab5 on early endosomes. EEA1 contains a calmodulin-binding IQ motif and cysteine rich finger motif necessary for its specific localization to the early endosomes. EEA1 has sequence homology to several yeast proteins that have been implicated in membrane trafficking, including Vps27, Fab1 and Vac1. Evidence suggests a possible role for EEA1 in mediating the regulatory effects of 3'-phosphoinositides on membrane trafficking.

REFERENCES

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2. Piper, R.C., et al. 1995. Vps27 controls vacuolar and endocytic traffic through a prevacuolar compartment in *Saccharomyces cerevisiae*. *J. Cell Biol.* 131: 603-617.
3. Yamamoto, A., et al. 1995. Novel PI(4)P 5-kinase homologue, Fab1p, essential for normal vacuole function and morphology in yeast. *Mol. Biol. Cell* 6: 525-539.
4. Mu, F.T., et al. 1995. EEA1, an early endosome-associated protein. EEA1 is a conserved α -helical peripheral membrane protein flanked by cysteine "fingers" and contains a calmodulin-binding IQ motif. *J. Biol. Chem.* 270: 13503-13511.
5. Bottger, G., et al. 1996. Rab 4 and Rab 7 define distinct nonoverlapping endosomal compartments. *J. Biol. Chem.* 271: 29191-29197.
6. Stenmark, H., et al. 1996. Endosomal localization of the autoantigen EEA1 is mediated by a zinc-binding FYVE finger. *J. Biol. Chem.* 271: 24048-24054.
7. Patki, V., et al. 1997. Identification of an early endosomal protein regulated by phosphatidylinositol 3-kinase. *Proc. Natl. Acad. Sci. USA* 94: 7326-7330.
8. D'Arrigo, A., et al. 1997. Microtubules are involved in bafilomycin A1-induced tubulation and Rab 5-dependent vacuolation of early endosomes. *Eur. J. Cell Biol.* 72: 95-103.

CHROMOSOMAL LOCATION

Genetic locus: Eea1 (mouse) mapping to 10 C2.

PRODUCT

EEA1 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see EEA1 shRNA Plasmid (m): sc-35264-SH and EEA1 shRNA (m) Lentiviral Particles: sc-35264-V as alternate gene silencing products.

For independent verification of EEA1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35264A, sc-35264B and sc-35264C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

EEA1 siRNA (m) is recommended for the inhibition of EEA1 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

EEA1 (G-4): sc-137130 is recommended as a control antibody for monitoring of EEA1 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor EEA1 gene expression knockdown using RT-PCR Primer: EEA1 (m)-PR: sc-35264-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.