

GAP1-InsP₄ BP siRNA (m): sc-39024

BACKGROUND

Human GAP1-InsP₄ BP, also designated Ras p21 protein activator (GTPase-activating protein) 3 [Ins(1,3,4,5)P₄-binding protein], is an 829-amino acid protein that binds phospholipids in both a calcium-dependent and -independent manner. GAP1, one of the Ras GTPase-activating protein families, comprises four distinct genes, including GAP1(m), GAP1-InsP₄ BP, MRASAL (murine Ras GTPase-activating-like) and KIAA0538. This family contains an N-terminal tandem C2 domain, a GAP-related domain and a C-terminal pleckstrin homology (PH) domain. The PH domains of GAP1-InsP₄ BP are essential for membrane targeting via binding of specific phospholipids. Following agonist-stimulated PtdIns(3,4,5)P(3) production, group I family PH domain containing proteins like GAP1-InsP₄ BP specifically bind inositol phosphates, which are subsequently targeted to the plasma membrane.

REFERENCES

- Cozier, G.E., et al. 2000. GAP1-InsP₄ BP contains a novel group I pleckstrin homology domain that directs constitutive plasma membrane association. *J. Biol. Chem.* 275: 28261-28268.
- Cozier, G., et al. 2000. Molecular modeling and site-directed mutagenesis of the inositol 1,3,4,5-tetrakisphosphate-binding pleckstrin homology domain from the Ras GTPase-activating protein GAP1-InsP₄ BP. *Biochem. J.* 349: 333-342.
- Online Mendelian Inheritance in Man, OMIM[™]. 2000. Johns Hopkins University, Baltimore, MD. MIM Number: 605182. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
- Minagawa, T., et al. 2001. Distinct phosphoinositide binding specificity of the GAP1 family proteins: characterization of the pleckstrin homology domains of MRASAL and KIAA0538. *Biochem. Biophys. Res. Commun.* 288: 87-90.
- LocusLink Report (LocusID: 22821). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: Rasa3 (mouse) mapping to 8 A1.1.

PRODUCT

GAP1-InsP₄ BP 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 GAP1-InsP₄ BP shRNA Plasmid (m): sc-39024-SH and GAP1-InsP₄ BP shRNA (m) Lentiviral Particles: sc-39024-V as alternate gene silencing products.

For independent verification of GAP1-InsP₄ BP (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-39024A, sc-39024B and sc-39024C.

PROTOCOLS

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

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

GAP1-InsP₄ BP siRNA (m) is recommended for the inhibition of GAP1-InsP₄ BP 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

GAP1-InsP₄ BP (E-9): sc-398283 is recommended as a control antibody for monitoring of GAP1-InsP₄ BP 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 GAP1-InsP₄ BP gene expression knockdown using RT-PCR Primer: GAP1-InsP₄ BP (m)-PR: sc-39024-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.