

Rabex-5 siRNA (h): sc-62920

BACKGROUND

Rabex-5 (Rab 5 GDP/GTP exchange factor), also known as RABGEF1, RAP1 or RABAPTIN-5-associated exchange factor for Rab 5, is a Rab guanine nucleotide exchange factor. Rabex-5 localizes to the cytoplasm and can associate with early endosomes. It consists of an N-terminal zinc finger domain, a GEF domain, an EET (early endosomal targeting) domain and a coiled-coil domain. The EET domain is important for the association of Rabex-5 with early endosomes and for the activation of Rab 5. Truncated Rabex-5 that is missing its EET domain can still function via an association with RABAPTIN-5. The Rabex-5/RABAPTIN-5 complex can target to early endosomes in a Rab 5-dependent manner through the binding of Rab5-GTP to RABAPTIN-5. *In vitro*, Rabex-5 exhibits GEF activity on its own, however, its association with RABAPTIN-5 increases its efficiency.

REFERENCES

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2. Tam, S.Y., et al. 2005. RabGEF1, a negative regulator of Ras signalling, mast cell activation and skin inflammation. *Novartis Found. Symp.* 271: 115-124
3. Penengo, L., et al. 2006. Crystal structure of the ubiquitin binding domains of Rabex-5 reveals two modes of interaction with ubiquitin. *Cell* 124: 1183-1195.
4. Lee, S., et al. 2006. Structural basis for ubiquitin recognition and autoubiquitination by Rabex-5. *Nat. Struct. Mol. Biol.* 13: 264-271.
5. Mattera, R., et al. 2006. The Rab5 guanine nucleotide exchange factor Rabex-5 binds ubiquitin (Ub) and functions as a Ub ligase through an atypical Ub-interacting motif and a zinc finger domain. *J. Biol. Chem.* 281: 6874-6883.
6. Zhu, H., et al. 2007. Rabaptin-5-independent membrane targeting and Rab5 activation by Rabex-5 in the cell. *Mol. Biol. Cell* 18: 4119-4128.
7. Delprato, A., et al. 2007. Structural basis for Rab GTPase activation by VPS9 domain exchange factors. *Nat. Struct. Mol. Biol.* 14: 406-412.
8. Hayakawa, A., et al. 2007. Evolutionarily conserved structural and functional roles of the FYVE domain. *Biochem. Soc. Symp.* 74: 95-105.

CHROMOSOMAL LOCATION

Genetic locus: RABGEF1 (human) mapping to 7q11.21.

PRODUCT

Rabex-5 siRNA (h) 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 Rabex-5 shRNA Plasmid (h): sc-62920-SH and Rabex-5 shRNA (h) Lentiviral Particles: sc-62920-V as alternate gene silencing products.

For independent verification of Rabex-5 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-62920A, sc-62920B and sc-62920C.

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

Rabex-5 siRNA (h) is recommended for the inhibition of Rabex-5 expression in human 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

Rabex-5 (A-7): sc-166611 is recommended as a control antibody for monitoring of Rabex-5 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 Rabex-5 gene expression knockdown using RT-PCR Primer: Rabex-5 (h)-PR: sc-62920-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.