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RUFY1 siRNA (m): sc-153172

BACKGROUND

RUFY1 (RUN and FYVE domain containing 1), also known as RABIP4 or ZFYVE12, is a 708 amino acid protein that localizes to the cytoplasm and the early endosome membrane. Highly expressed in testis, lung, brain and kidney, RUFY1 functions to bind phosphatidylinositol 3-phosphate-containing phospholipid vesicles and, via this interaction, participates in early endosomal trafficking. RUFY1 contains one RUN domain and one FYVE-type zinc finger through which it mediates its ability to bind phosphatidylinositol 3-phosphate. Upon DNA damage, RUFY1 may be phosphorylated by Atm or ATR. Additionally, the phosphorylation of Tyr 389 and/or Tyr 400 residues on human RUFY1 is thought to be necessary for endosomal localization. Three isoforms of RUFY1 exist due to alternative splicing events.

REFERENCES

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- Fouraux, M.A., et al. 2004. RABIP4' is an effector of Rab5 and Rab4 and regulates transport through early endosomes. *Mol. Biol. Cell* 15: 611-624.
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- Vukmirica, J., et al. 2006. The Rab4A effector protein RABIP4 is involved in migration of NIH 3T3 fibroblasts. *J. Biol. Chem.* 281: 36360-36368.
- Mari, M., et al. 2006. The Rab4 effector RABIP4 plays a role in the endocytotic trafficking of Glut 4 in 3T3-L1 adipocytes. *J. Cell Sci.* 119: 1297-1306.

CHROMOSOMAL LOCATION

Genetic locus: *Rufy1* (mouse) mapping to 11 B1.3.

PRODUCT

RUFY1 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 RUFY1 shRNA Plasmid (m): sc-153172-SH and RUFY1 shRNA (m) Lentiviral Particles: sc-153172-V as alternate gene silencing products.

For independent verification of RUFY1 (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-153172A, sc-153172B and sc-153172C.

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

RUFY1 siRNA (m) is recommended for the inhibition of RUFY1 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

RUFY1 (A-4): sc-398740 is recommended as a control antibody for monitoring of RUFY1 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 RUFY1 gene expression knockdown using RT-PCR Primer: RUFY1 (m)-PR: sc-153172-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.