

JRAB siRNA (h): sc-89784

BACKGROUND

JRAB (junctional Rab13-binding protein, MICAL-like protein 2) is a 904 amino acid protein with one CH (calponin-homology) domain and one LIM zinc-binding domain. JRAB has been shown to interact with Rab13 and Rab8 to facilitate cellular transport of claudin-1, occludin, and E-cadherin. This interaction is vital for the coordination of the assembly of tight junctions (TJs) and adherens junctions (AJs). Dynamic turnover (endocytic recycling) of cell-to-cell AJs and TJs is essential for epithelial morphogenesis during normal development and differentiation. The endocytic recycling of occludin and claudin proteins is part of an ongoing process of restructuring and maintaining cell junctions, especially at TJs. JRAB and Rab13 have also been implicated in the carcinoma metastasis event of epithelial cell scattering. This event shows Rab13 and JRAB colocalizing with F-Actin in lamellipodial structures prior to cell scattering.

REFERENCES

1. Terai, T., et al. 2006. JRAB/MICAL-L2 is a junctional Rab13-binding protein mediating the endocytic recycling of occludin. *Mol. Biol. Cell* 17: 2465-2475.
2. Nishimura, N. and Sasaki, T. 2008. Regulation of epithelial cell adhesion and repulsion: role of endocytic recycling. *J. Med. Invest.* 55: 9-16.
3. Nishimura, N. and Sasaki, T. 2008. Identification and characterization of JRAB/MICAL-L2, a junctional Rab13-binding protein. *Meth. Enzymol.* 438: 141-153.
4. Yamamura, R., et al. 2008. The interaction of JRAB/MICAL-L2 with Rab8 and Rab13 coordinates the assembly of tight junctions and adherens junctions. *Mol. Biol. Cell* 19: 971-983.
5. Nakatsuji, H., et al. 2008. Involvement of actinin-4 in the recruitment of JRAB/MICAL-L2 to cell-cell junctions and the formation of functional tight junctions. *Mol. Cell. Biol.* 28: 3324-3335.
6. Kanda, I., et al. 2008. Involvement of Rab13 and JRAB/MICAL-L2 in epithelial cell scattering. *Oncogene* 27: 1687-1695.
7. Nishimura, N. and Sasaki, T. 2009. Rab family small G proteins in regulation of epithelial apical junctions. *Front. Biosci.* 14: 2115-2129.
8. Sakane, A., et al. 2010. Rab13 regulates neurite outgrowth in PC-12 cells through its effector protein, JRAB/MICAL-L2. *Mol. Cell. Biol.* 30: 1077-1087.

CHROMOSOMAL LOCATION

Genetic locus: MICALL2 (human) mapping to 7p22.3.

PRODUCT

JRAB siRNA (h) is a pool of 2 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 JRAB shRNA Plasmid (h): sc-89784-SH and JRAB shRNA (h) Lentiviral Particles: sc-89784-V as alternate gene silencing products.

For independent verification of JRAB (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-89784A and sc-89784B.

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

JRAB siRNA (h) is recommended for the inhibition of JRAB 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

JRAB (F-5): sc-376675 is recommended as a control antibody for monitoring of JRAB 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 JRAB gene expression knockdown using RT-PCR Primer: JRAB (h)-PR: sc-89784-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.