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CRB1 siRNA (h): sc-44481

BACKGROUND

The transmembrane protein Crumbs plays a crucial role in epithelial cell polarity and photoreceptor development in *Drosophila melanogaster* embryos, but the first identified human homologue, CRB1, is only expressed in retina and some parts of the brain, leaving room for another homologue to function in epithelial tissues. Leber congenital amaurosis or progressive retinitis pigmentosa are caused by loss of CRB1 function. A second homologue, CRB3, fills the gap, showing expression in epithelial tissues as well as skeletal muscles. CRB3 shares a cytoplasmic domain with other Crumbs proteins, but contains only a very short extracellular domain, through which it interacts with Par6, a regulator of epithelial polarity and tight junction formation. Thus, this specialized isoform provides a connection between apical membrane formation and tight junction regulation.

REFERENCES

1. Roh, M.H., et al. 2002. The Maguk protein, Pals1, functions as an adapter, linking mammalian homologues of Crumbs and Discs Lost. *J. Cell Biol.* 157: 161-172.
2. Makarova, O., et al. 2003. Mammalian Crumbs3 is a small transmembrane protein linked to protein associated with Lin-7 (Pals1). *Gene* 302: 21-29.
3. van de Pavert, S.A., et al. 2004. Crumbs homologue 1 is required for maintenance of photoreceptor cell polarization and adhesion during light exposure. *J. Cell Sci.* 117: 4169-4177.
4. Lemmers C, Michel, D., et al. 2004. CRB3 binds directly to Par6 and regulates the morphogenesis of the tight junctions in mammalian epithelial cells. *Mol. Biol. Cell* 15: 1324-1333.

CHROMOSOMAL LOCATION

Genetic locus: CRB1 (human) mapping to 1q31.3.

PRODUCT

CRB1 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 CRB1 shRNA Plasmid (h): sc-44481-SH and CRB1 shRNA (h) Lentiviral Particles: sc-44481-V as alternate gene silencing products.

For independent verification of CRB1 (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-44481A, sc-44481B and sc-44481C.

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

CRB1 siRNA (h) is recommended for the inhibition of CRB1 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CRB1 gene expression knockdown using RT-PCR Primer: CRB1 (h)-PR: sc-44481-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.