

Recoverin siRNA (h): sc-40905

BACKGROUND

Light triggers the phototransduction cascade by activating the visual pigment rhodopsin. Phosphorylation of Rho by rhodopsin kinase is required for the recovery of sensitivity after intense illumination. Ca^{2+} ions act through Ca^{2+} -binding proteins and are implicated in the desensitization of phototransduction. Recoverin is implicated in the regulation of rhodopsin kinase activity that contributes to the adaptation to background illumination in retinal photoreceptor cells. Recoverin, a Ca^{2+} -binding photoreceptor protein, is recognized as an autoantigen of cancer-associated retinopathy (CAR), which is a rare paraneoplastic neurological syndrome characterized by the degeneration of retinal photoreceptors and associated with small-cell lung cancer. Recoverin is a heterogeneously myristoylated protein that inhibits rhodopsin kinase by inhibiting its phosphorylation. Ca^{2+} is required for Recoverin to bind rhodopsin kinase. In addition, the binding of Recoverin-rhodopsin kinase is weakened by autophosphorylation of the kinase and is strengthened by the presence of ADP. Upon accommodating two Ca^{2+} ions, Recoverin extrudes a myristoyl group and associates with the lipid bilayer membrane.

REFERENCES

1. Thirkill, C.E., et al. 1992. The cancer-associated retinopathy antigen is a Recoverin-like protein. *Invest. Ophthalmol. Vis. Sci.* 33: 2768-2772.
2. Matsubara, S., et al. 1996. Expression of a photoreceptor protein, Recoverin, as a cancer-associated retinopathy autoantigen in human lung cancer cell lines. *Br. J. Cancer* 74: 1419-1422.
3. Satpaev, D.K., et al. 1998. Autophosphorylation and ADP regulate the Ca^{2+} -dependent interaction of Recoverin with rhodopsin kinase. *Biochemistry* 37: 10256-10262.
4. Otto-Bruc, A.E., et al. 1998. Phosphorylation of photolyzed rhodopsin in calcium-insensitive in retina permeabilized by α -toxin. *Proc. Natl. Acad. Sci. USA* 95: 15014-15019.
5. Kawamura, S. 1999. Calcium-dependent regulation of rhodopsin phosphorylation. *Novartis Found. Symp.* 224: 208-218.
6. Ozawa, T., et al. 2000. How can Ca^{2+} selectively activate recoverin in the presence of Mg^{2+} ? Surface plasmon resonance and FT-1R spectroscopic studies. *Biochemistry* 39: 14495-14503.

CHROMOSOMAL LOCATION

Genetic locus: RCVRN (human) mapping to 17p13.1.

PRODUCT

Recoverin 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Recoverin shRNA Plasmid (h): sc-40905-SH and Recoverin shRNA (h) Lentiviral Particles: sc-40905-V as alternate gene silencing products.

For independent verification of Recoverin (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-40905A, sc-40905B and sc-40905C.

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 μl of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μl of RNase-free water makes a 10 μM solution in a 10 μM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Recoverin siRNA (h) is recommended for the inhibition of Recoverin 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 μM in 66 μl . 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

Recoverin (6A55CD6): sc-53520 is recommended as a control antibody for monitoring of Recoverin 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 Recoverin gene expression knockdown using RT-PCR Primer: Recoverin (h)-PR: sc-40905-PR (20 μl). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

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.