

LRRC8D siRNA (h): sc-88508

BACKGROUND

The leucine-rich repeat (LRR) is a 20-30 amino acid motif that forms a hydrophobic α/β horseshoe fold, allowing it to accommodate several leucine residues within a tightly packed core. All LRRs contain a variable segment and a highly conserved segment, the latter of which accounts for 11 or 12 residues of the entire LRR motif. The primary function of this motif is to provide a versatile structural framework to mediate the formation of protein-protein interactions. LRRs are present in a variety of proteins with diverse structure and function, including innate immunity and nervous system development. Several human diseases are associated with mutations in genes encoding LRR-containing proteins. LRRC8D (leucine-rich repeat-containing protein 8D, also known as LRRC5, is a 858 amino acid multi-pass membrane protein that contains thirteen LRRs.

REFERENCES

1. Gomi, F., Imaizumi, K., Yoneda, T., Taniguchi, M., Mori, Y., Miyoshi, K., Hitomi, J., Fujikado, T., Tano, Y. and Tohyama, M. 2000. Molecular cloning of a novel membrane glycoprotein, pal, specifically expressed in photoreceptor cells of the retina and containing leucine-rich repeat. *J. Neurosci.* 20: 3206-3213.
2. Kobe, B. and Kajava, A.V. 2001. The leucine-rich repeat as a protein recognition motif. *Curr. Opin. Struct. Biol.* 11: 725-732.
3. Hofman, P., Hoyng, P., vanderWerf, F., Vrensen, G.F. and Schlingemann, R.O. 2001. Lack of blood-brain barrier properties in microvessels of the prelaminar optic nerve head. *Invest. Ophthalmol. Vis. Sci.* 42: 895-901.
4. Hughes, J.M., Brink, A., Witmer, A.N., Hanraads-de Riemer, M., Klaassen, I. and Schlingemann, R.O. 2004. Vascular leucocyte adhesion molecules unaltered in the human retina in diabetes. *Br. J. Ophthalmol.* 88: 566-572.
5. Kuiper, E.J., Witmer, A.N., Klaassen, I., Oliver, N., Goldschmeding, R. and Schlingemann, R.O. 2004. Differential expression of connective tissue growth factor in microglia and pericytes in the human diabetic retina. *Br. J. Ophthalmol.* 88: 1082-1087.
6. Matsushima, N., Tachi, N., Kuroki, Y., Enkhbayar, P., Osaki, M., Kamiya, M. and Kretsinger, R.H. 2005. Structural analysis of leucine-rich-repeat variants in proteins associated with human diseases. *Cell. Mol. Life Sci.* 62: 2771-2791.

CHROMOSOMAL LOCATION

Genetic locus: LRRC8D (human) mapping to 1p22.2.

PRODUCT

LRRC8D 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 LRRC8D shRNA Plasmid (h): sc-88508-SH and LRRC8D shRNA (h) Lentiviral Particles: sc-88508-V as alternate gene silencing products.

For independent verification of LRRC8D (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-88508A, sc-88508B and sc-88508C.

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

LRRC8D siRNA (h) is recommended for the inhibition of LRRC8D 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

LRRC8D (A-12): sc-515070 is recommended as a control antibody for monitoring of LRRC8D 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 MarkerTM 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 LRRC8D gene expression knockdown using RT-PCR Primer: LRRC8D (h)-PR: sc-88508-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.