SANTA CRUZ BIOTECHNOLOGY, INC.

RYK siRNA (m): sc-72222

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The Power to Question

BACKGROUND

RYK (related to receptor tyrosine kinase) is a chemorepulsive axon guidance receptor required for establishing major axon tracts, such as the corticospinal tract and the corpus callosum, in the developing nervous system. RYK belongs to the RTK family and is ubiquitously expressed in both developing and adult tissues. Compared to other RTK family members, RYK has a relatively short extracellular domain. In addition, RYK contains two N-terminal leucine-rich motifs that resemble the N-terminal domain of WIF-1. RYK functions as a Wnt receptor and produces a repulsive guidance signal upon binding of Wnt-1, 3, 3a or 5a to its WIF-like domain. The loss of functional RYK results in the formation of contralateral axon bundles and the disruption of cardiac, skeletal and craniofacial development, ultimately resulting in perinatal death. RYK is also strongly expressed in malignant ovarian tumors and may play a role in tumorigenesis.

REFERENCES

1. Hovens, C.M., et al. 1993. RYK, a receptor tyrosine kinase-related molecule with unusual kinase domain motifs. Proc. Natl. Acad. Sci. USA 89: 11818-11822.
2. Katso, R.M., et al. 1999. Functional analysis of H-RYK, an atypical member of the receptor tyrosine kinase family. Mol. Cell. Biol. 19: 6427-6440.
3. Halford, M.M., et al. 2000. RYK-deficient mice exhibit craniofacial defects associated with perturbed Eph receptor crosstalk. Nat. Genet. 25: 414-418.
4. Halford, M.M. and Stacker, S.A. 2001. Revelations of the RYK receptor. Bioessays 23: 34-45.
5. Trivier, E. and Ganesan, T.S. 2002. RYK, a catalytically inactive receptor tyrosine kinase, associates with EphB2 and EphB3 but does not interact with AF-6. J. Biol. Chem. 277: 23037-23043.
6. Lu, W., et al. 2004. Mammalian RYK is a Wnt co-receptor required for stimulation of neurite outgrowth. Cell 119: 97-108.
7. Liu, Y., et al. 2005. RYK-mediated Wnt repulsion regulates posterior-directed growth of corticospinal tract. Nat. Neurosci. 8: 1151-1159.

CHROMOSOMAL LOCATION

Genetic locus: Ryk (mouse) mapping to 9 F1.

PRODUCT

RYK 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RYK shRNA Plasmid (m): sc-72222-SH and RYK shRNA (m) Lentiviral Particles: sc-72222-V as alternate gene silencing products.

For independent verification of RYK (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-72222A, sc-72222B and sc-72222C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

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

RYK (F35 P7 D7 F5): sc-83082 is recommended as a control antibody for monitoring of RYK 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 RYK gene expression knockdown using RT-PCR Primer: RYK (m)-PR: sc-72222-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.