

mGluR-5 siRNA (r): sc-270671

BACKGROUND

The mGluR proteins (metabotropic Glutamate Receptors) are members of the G protein-coupled receptor family and are functionally and pharmacologically distinct from the GluR proteins (ionotropic Glutamate Receptors). The eight currently known mGluR proteins are mediated by two G proteins with opposing regulation of adenylate cyclase pathways. The activities of mGluR1 and mGluR5 are mediated by a G protein that activates a phosphatidylinositol-calcium second messenger system and generates a calcium-activated chloride current. The remainder of the eight sub-types of mGluR have an activity mediated by a G protein that inhibits adenylate cyclase activity. mGluR-5, which can interact with SIAH-1, RyR-1, RyR-2, ITPR1, Shank 1, Shank 3 and GRASP, acts as a receptor for glutamate. The PPXXf motif of mGluR-5 binds to HOM1, HOM2 and HOM3.

REFERENCES

1. Minakami, R., et al. 1993. A variant of metabotropic glutamate receptor subtype 5: an evolutionally conserved insertion with no termination codon. *Biochem. Biophys. Res. Commun.* 194: 622-627.
2. Minakami, R., et al. 1994. Molecular cloning and the functional expression of two isoforms of human metabotropic glutamate receptor subtype 5. *Biochem. Biophys. Res. Commun.* 199: 1136-1143.
3. Yang, L., et al. 2004. A Novel Ca^{2+} -independent signaling pathway to extracellular signal-regulated protein kinase by coactivation of NMDA receptors and metabotropic glutamate receptor 5 in neurons. *J. Neurosci.* 24: 10846-10857.
4. Molina-Hernández, M., et al. 2006. Antidepressant-like and anxiolytic-like actions of the mGlu5 receptor antagonist MTEP, microinjected into lateral septal nuclei of male Wistar rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 30: 1129-1135.
5. Sen, M. and Gleason, E. 2006. Immunolocalization of metabotropic glutamate receptors 1 and 5 in the synaptic layers of the chicken retina. *Vis. Neurosci.* 23: 221-231.
6. Ametamey, S.M., et al. 2006. Radiosynthesis and preclinical evaluation of ^{11}C -ABP688 as a probe for imaging the metabotropic glutamate receptor subtype 5. *J. Nucl. Med.* 47: 698-705.

CHROMOSOMAL LOCATION

Genetic locus: Grm5 (rat) mapping to 1q32.

PRODUCT

mGluR-5 siRNA (r) 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 mGluR-5 shRNA Plasmid (r): sc-270671-SH and mGluR-5 shRNA (r) Lentiviral Particles: sc-270671-V as alternate gene silencing products.

For independent verification of mGluR-5 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270671A, sc-270671B and sc-270671C.

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

mGluR-5 siRNA (r) is recommended for the inhibition of mGluR-5 expression in rat 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

mGluR-5 (1B3): sc-293442 is recommended as a control antibody for monitoring of mGluR-5 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 mGluR-5 gene expression knockdown using RT-PCR Primer: mGluR-5 (r)-PR: sc-270671-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ 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.