

Gephyrin siRNA (h): sc-35464

BACKGROUND

The sub-membrane region at the postsynaptic membrane contains a number of proteins critical for receptor targeting. Gephyrin is a microtubule-associated protein highly expressed in brain and localized to neuronal post-synaptic membranes. Gephyrin is essential for the postsynaptic localization of the inhibitory glycine receptor and is thought to anchor the receptor to subsynaptic microtubules. The protein is expressed in most mammalian tissues with predominant expression in brain. At least five additional splice variants of Gephyrin have been identified in rat and human brain tissue.

REFERENCES

1. Prior, P., et al. 1992. Primary structure and alternative splice variants of Gephyrin, a putative glycine receptor-Tubulin linker protein. *Neuron* 8: 1161-1170.
2. Takagi, T., et al. 1992. Coexpression of the receptor-associated protein Gephyrin changes the ligand binding affinities of α 2 glycine receptors. *FEBS Lett.* 303: 178-180.
3. Kirsch, J., et al. 1993. Distribution of Gephyrin transcripts in the adult and developing rat brain. *Eur. J. Neurosci.* 5: 1109-1117.
4. Meyer, G., et al. 1995. Identification of a Gephyrin binding motif on the glycine receptor β subunit. *Neuron* 15: 563-572.
5. Vannier, C., et al. 1997. Biology of the postsynaptic glycine receptor. *Int. Rev. Cytol.* 176: 201-244.
6. Kawasaki, B.T., et al. 1997. Variants of the receptor/channel clustering molecule Gephyrin in brain: distinct distribution patterns, developmental profiles, and proteolytic cleavage by calpain. *J. Neurosci. Res.* 49: 381-388.

CHROMOSOMAL LOCATION

Genetic locus: GPHN (human) mapping to 14q23.3.

PRODUCT

Gephyrin 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 Gephyrin shRNA Plasmid (h): sc-35464-SH and Gephyrin shRNA (h) Lentiviral Particles: sc-35464-V as alternate gene silencing products.

For independent verification of Gephyrin (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-35464A, sc-35464B and sc-35464C.

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

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

Gephyrin (G-6): sc-25311 is recommended as a control antibody for monitoring of Gephyrin 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 Gephyrin gene expression knockdown using RT-PCR Primer: Gephyrin (h)-PR: sc-35464-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.