SANTA CRUZ BIOTECHNOLOGY, INC.

GSK-3α siRNA (r): sc-270583

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

Glycogen synthase kinase 3, or GSK-3, is a serine/threonine, proline-directed kinase involved in a diverse array of signaling pathways, including glycogen synthesis and cellular adhesion, and has been implicated in Alzheimer's disease. Two forms of GSK-3, designated GSK-3α and GSK-3β, have been identified and differ in their subcellular localization. Tau, a microtubule-binding protein which serves to stabilize microtubules in growing axons, is found to be hyper-phosphorylated in paired helical filaments (PHF), the major fibrous component of neurofibrillary lesions associated with Alzheimer's disease. Hyperphosphorylation of Tau is thought to be the critical event leading to the assembly of PHF. Six Tau protein isoforms have been identified, all of which are phosphorylated by GSK-3. This presents the possibility that miscues in GSK-3 signaling contribute to the onset of Alzheimer's disease.

REFERENCES

1. Pugazhenthi, S., et al. 1995. Regulation of glycogen synthase activation in isolated hepatocytes. Mol. Cell. Biochem. 149-150: 95-101.
2. Pelech, S.L. 1995. Networking with proline-directed protein kinases implicated in Tau phosphorylation. Neurobiol. Aging 16: 247-256.
3. Hoshi, M., et al. 1995. Different localization of Tau protein kinase I/glycogen synthase kinase-3 β from glycogen synthase kinase-3 α in cerebellum mitochondria. J. Biochem. 118: 683-685.
4. Sperber, B.R., et al. 1995. Glycogen synthase kinase-3 β phosphorylates Tau protein at multiple sites in intact cells. Neurosci. Lett. 197: 149-153.
5. Rubinfeld, B., et al. 1996. Binding of BSK3β to the APC-β-catenin complex and regulation of complex assembly. Science 272: 1023-1026.
6. Black, M.M., et al. 1996. Tau is enriched on dynamic microtubules in the distal region of growing axons. J. Neurosci. 16: 3601-3619.
7. Singh, T.J., et al. 1996. Differential phosphorylation of human Tau isoforms containing three repeats by several protein kinases. Arch. Biochem. Biophys. 328: 43-50.
8. Hoshi, M., et al. 1996. Regulation of mitochondrial pyruvate dehydrogenase activity by Tau protein kinase I/glycogen synthase kinase 3β in brain. Proc. Natl. Acad. Sci. USA 93: 2719-2723.

CHROMOSOMAL LOCATION

Genetic locus: Gsk3a (rat) mapping to 1q21.

PRODUCT

GSK-3α 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GSK-3α shRNA Plasmid (r): sc-270583-SH and GSK-3α shRNA (r) Lentiviral Particles: sc-270583-V as alternate gene silencing products.

For independent verification of GSK-3α (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-270583A, sc-270583B and sc-270583C.

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

GSK-3α siRNA (r) is recommended for the inhibition of GSK-3α 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 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

GSK-3α (H-12): sc-5264 is recommended as a control antibody for monitoring of GSK-3α 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 GSK-3α gene expression knockdown using RT-PCR Primer: GSK-3α (r)-PR: sc-270583-PR (20 µl). 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.