

GFAP siRNA (m): sc-35466

BACKGROUND

Glial fibrillary acidic protein, or GFAP, is an intermediate filament (IF) protein belonging to the type III subclass of IF proteins. Like other IF proteins, GFAP is composed of an amino terminal head domain, a central rod domain and a carboxy terminal tail domain. GFAP is specifically found in astroglia, a cell type which is highly responsive to neurologic insults. Astrogliosis is found to be a result of mechanical trauma, AIDS dementia, prion infection and inflammatory demyelination diseases, and is accompanied by an increase in GFAP expression. GFAP is an immunohistochemical marker for localizing benign astrocyte and neoplastic cells of glial origin in the central nervous system.

REFERENCES

1. Herpers, M.J., Ramaekers, F.C., Aldewireldt, J., Moesker, O. and Slooff, J. 1986. Co-expression of glial fibrillary acidic protein- and Vimentin-type intermediate filaments in human astrocytomas. *Acta Neuropathol.* 70: 333-339.
2. Van Muijen, G.N., Ruiter, D.J. and Warnaar, S.O. 1987. Coexpression of intermediate filament polypeptides in human fetal and adult tissues. *Lab. Invest.* 57: 359-369.
3. McLendon, R.E. and Bigner, D.D. 1994. Immunohistochemistry of the glial fibrillary acidic protein: basic and applied considerations. *Brain Pathol.* 4: 221-228.
4. Eng, L.F. and Ghirnikar, R.S. 1994. GFAP and astrogliosis. *Brain Pathol.* 4: 229-237.
5. Inagaki, M., Nakamura, Y., Takeda, M., Nishimura, T. and Inagaki, N. 1994. Glial fibrillary acidic protein: dynamic property and regulation by phosphorylation. *Brain Pathol.* 4: 239-243.

CHROMOSOMAL LOCATION

Genetic locus: Gfap (mouse) mapping to 11 E1.

PRODUCT

GFAP 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GFAP shRNA Plasmid (m): sc-35466-SH and GFAP shRNA (m) Lentiviral Particles: sc-35466-V as alternate gene silencing products.

For independent verification of GFAP (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-35466A, sc-35466B and sc-35466C.

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.

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

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

GFAP (2E1): sc-33673 is recommended as a control antibody for monitoring of GFAP 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 GFAP gene expression knockdown using RT-PCR Primer: GFAP (m)-PR: sc-35466-PR (20 μ l, 467 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.