SANTA CRUZ BIOTECHNOLOGY, INC.

MBP siRNA (h): sc-35871

BACKGROUND

Myelin basic protein (MBP) is the major extrinsic membrane protein of central nervous system myelin. MBP phosphorylation at Threonine 125 is a complex regulatory process that modulates the contribution of MBP to the stability of the myelin sheath. Mitogen-activated protein kinases modulate MBP phosphorylation during myelinogenesis and in the demyelinating disease multiple sclerosis. MBP phosphorylation is regulated by high-frequency stimulation but not low-frequency stimulation of the alveus, the myelinated output fibers of the hippocampus. It has been proposed that during periods of increased neuronal activity, calcium activates axonal nitric oxide synthase, which generates the intercellular messengers nitric oxide and superoxide and regulates the phosphorylation state of MBP by MAPK.

REFERENCES

1. Fraser, P.E. and Deber, C.M. 1985. Structure and function of the proline-rich region of myelin basic protein. Biochemistry 24: 4593-4598.
2. Potter, N.T., et al. 1986. Identification of an antigenic determinant within the phylogenetically conserved triprolyl region of myelin basic protein. J. Immunol. 136: 516-520.
3. Persaud, R., et al. 1988. The glycosylation of human myelin basic protein at Threonines 95 and 98 occurs sequentially. Biochim. Biophys. Acta 966: 357-361.
4. Yon, M., et al. 1996. Identification of a mitogen-activated protein kinase site in human myelin basic protein *in situ*. J. Neuroimmunol. 65: 55-59.
5. Atkins, C.M., et al. 1999. Regulation of myelin basic protein phosphorylation by mitogen-activated protein kinase during increased action potential firing in the hippocampus. J. Neurochem. 73: 1090-1097.
6. Bielekova, B., et al. 2000. Encephalitogenic potential of the myelin basic protein peptide (amino acids 83-99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. Nat. Med. 6: 1167-1175.
7. Chignola, R., et al. 2000. Expression of myelin basic protein (MBP) epitopes in human non-neural cells revealed by two anti-MBP IgM monoclonal antibodies. Clin. Exp. Immunol. 122: 429-436.

CHROMOSOMAL LOCATION

Genetic locus: MBP (human) mapping to 18q23.

PRODUCT

MBP 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MBP shRNA Plasmid (h): sc-35871-SH and MBP shRNA (h) Lentiviral Particles: sc-35871-V as alternate gene silencing products.

For independent verification of MBP (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-35871A, sc-35871B and sc-35871C.

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

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

MBP (F-6): sc-271524 is recommended as a control antibody for monitoring of MBP 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 MBP gene expression knockdown using RT-PCR Primer: MBP (h)-PR: sc-35871-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.