

DUSP8 siRNA (h): sc-60556

BACKGROUND

Mitogen-activated protein (MAP) kinases are a large class of proteins involved in signal transduction pathways that are activated by a range of stimuli and mediate a number of physiological and pathological changes in the cell. Dual specificity phosphatases (DSPs) are a subclass of the protein tyrosine phosphatase (PTP) gene superfamily, which are selective for dephosphorylating critical phosphothreonine and phosphotyrosine residues within MAP kinases. DSP gene expression is induced by a host of growth factors and/or cellular stresses, thereby negatively regulating MAP kinase superfamily members including MAPK/ERK, SAPK/JNK and p38. DUSP8 inactivates SAPK/JNK and p38, is expressed predominantly in the adult brain, heart, and skeletal muscle. It localizes in the cytoplasm, and is induced by nerve growth factor and Insulin. DUSP8 may play a role in pathophysiological mechanisms leading to development of alcohol dependence.

REFERENCES

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2. Martell, K.J., Seasholtz, A.F., Kwak, S.P., Clemens, K.K. and Dixon, J.E. 1995. hVH-5: a protein tyrosine phosphatase abundant in brain that inactivates mitogen-act protein kinase. *J. Neurochem.* 65: 1823-1833.
3. Sun, H. 1998. Functional studies of dual-specificity phosphatases. *Methods Mol. Biol.* 84: 307-318.
4. Camps, M., Nichols, A. and Arkinstall, S. 2000. Dual specificity phosphatases: a gene family for control of MAP kinase function. *FASEB J.* 14: 6-16.
5. Hink, R.L., Hokanson, J.E., Shah, I., Long, J.C., Goldman, D. and Sikela, J.M. 2003. Investigation of DUSP8 and CALCA in alcohol dependence. *Addict. Biol.* 8: 305-312.

CHROMOSOMAL LOCATION

Genetic locus: DUSP8 (human) mapping to 11p15.5.

PRODUCT

DUSP8 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 DUSP8 shRNA Plasmid (h): sc-60556-SH and DUSP8 shRNA (h) Lentiviral Particles: sc-60556-V as alternate gene silencing products.

For independent verification of DUSP8 (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-60556A, sc-60556B and sc-60556C.

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

DUSP8 siRNA (h) is recommended for the inhibition of DUSP8 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

DUSP8 (B-9): sc-271250 is recommended as a control antibody for monitoring of DUSP8 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 DUSP8 gene expression knockdown using RT-PCR Primer: DUSP8 (h)-PR: sc-60556-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.