

# DARPP-32 siRNA (m): sc-35174

## BACKGROUND

Dopaminergic signaling pathways, which are essential for multiple brain functions, are abnormal in several neurological disorders, such as schizophrenia, Parkinson's disease and drug abuse. DARPP-32 (for Dopamine and adenosine 3',5'-monophosphate-regulated phosphoprotein) is abundant in neurons that receive dopaminergic input. Activation of PKA and the consequent phosphorylation of DARPP-32 on threonine occurs in response to Dopamine acting upon D1-like receptors. Dopamine interaction with D2-like receptors results in the inhibition of PKA activation, the activation of protein phosphatase 2B and the consequent dephosphorylation of DARPP-32. Neurotransmitters other than Dopamine may also be able to stimulate the phosphorylation or dephosphorylation of DARPP-32. Phosphorylated DARPP-32 is a potent inhibitor of PP-1.

## REFERENCES

1. Walaas, S.I., et al. 1984. DARPP-32, a Dopamine- and adenosine 3':5'-monophosphate-regulated phosphoprotein enriched in Dopamine-innervated brain regions. I. Regional and cellular distribution in the rat brain. *J. Neurosci.* 4: 84-98.
2. Hemmings, H.C., Jr., et al. 1984. DARPP-32, a Dopamine- and adenosine 3':5'-monophosphate-regulated neuronal phosphoprotein. I. Amino acid sequence around the phosphorylated threonine. *J. Biol. Chem.* 259: 14486-14490.
3. Hemmings, H.C., Jr., et al. 1984. DARPP-32, a Dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1. *Nature* 310: 503-505.
4. Nishi, A., et al. 1997. Bidirectional regulation of DARPP-32 phosphorylation by Dopamine. *J. Neurosci.* 17: 8147-8155.
5. Fienberg, A.A., et al. 1998. DARPP-32: regulator of the efficacy of dopaminergic neuro-transmission. *Science* 281: 838-842.
6. Greengard, P., et al. 1998. The DARPP-32/protein phosphatase-1 cascade: a model for signal integration. *Brain Res. Rev.* 26: 274-284.

## CHROMOSOMAL LOCATION

Genetic locus: Ppp1r1b (mouse) mapping to 11 D.

## PRODUCT

DARPP-32 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DARPP-32 shRNA Plasmid (m): sc-35174-SH and DARPP-32 shRNA (m) Lentiviral Particles: sc-35174-V as alternate gene silencing products.

For independent verification of DARPP-32 (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-35174A, sc-35174B and sc-35174C.

## PROTOCOLS

See our web site at [www.scbt.com](http://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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

DARPP-32 siRNA (m) is recommended for the inhibition of DARPP-32 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  $\mu$ M in 66  $\mu$ 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

DARPP-32 (H-3): sc-271111 is recommended as a control antibody for monitoring of DARPP-32 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 DARPP-32 gene expression knockdown using RT-PCR Primer: DARPP-32 (m)-PR: sc-35174-PR (20  $\mu$ 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.