

WNK3 siRNA (h): sc-39258

BACKGROUND

The protein kinase superfamily contains over a thousand proteins in 57 sub-families that all share a catalytic core of 250-300 amino acids organized in two domains. WNK, for "with no lysine (K)", kinases are serine-threonine protein kinases that contain a cysteine residue in place of a lysine residue in a family of proteins that traditionally contain a lysine following a short string of hydrophobic residues. WNK kinases contain a lysine upstream of the traditional position, within a glycine string. This lysine functions as an anchor and orients ATP through interactions with the α and β phosphoryl groups. The catalytic domains of WNK2, WNK3 and WNK4 are 95% homologous to WNK1. The human WNK1 gene encodes a 2,382 amino acid protein that is primarily expressed in heart, kidney, muscle and distal nephron. The human WNK3 gene encodes a protein that is primarily expressed in brain; the human WNK4 gene encodes a 1,243 amino acid protein that is expressed in kidney. Aberrant function of WNK kinases and their associated signaling pathways are implicated in hypertension, increased renal salt reabsorption and impaired K^+ and H^+ excretion.

REFERENCES

1. Xu, B., et al. 2000. WNK1, a novel mammalian serine/threonine protein kinase lacking the catalytic lysine in subdomain II. *J. Biol. Chem.* 275: 16795-16801.
2. Verissimo, F., et al. 2001. WNK kinases, a novel protein kinase subfamily in multi-cellular organisms. *Oncogene* 20: 5562-5569.
3. Wilson, F.H., et al. 2001. Human hypertension caused by mutations in WNK kinases. *Science* 293: 1107-1112.
4. Xu, B.E., et al. 2002. Regulation of WNK1 by an autoinhibitory domain and autophosphorylation. *J. Biol. Chem.* 277: 48456-48462.
5. Hollenberg, N.K. 2002. Human hypertension caused by mutations in WNK kinases. *Curr. Hypertens. Rep.* 4: 267.
6. Nakamichi, N., et al. 2002. Compilation and characterization of a novel WNK family of protein kinases in *Arabidopsis thaliana* with reference to circadian rhythms. *Biosci. Biotechnol. Biochem.* 66: 2429-2436.
7. Yang, C.L., et al. 2003. WNK kinases regulate thiazide-sensitive Na-Cl cotransport. *J. Clin. Invest.* 111: 1039-1045.

CHROMOSOMAL LOCATION

Genetic locus: WNK3 (human) mapping to Xp11.22.

PRODUCT

WNK3 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 WNK3 shRNA Plasmid (h): sc-39258-SH and WNK3 shRNA (h) Lentiviral Particles: sc-39258-V as alternate gene silencing products.

For independent verification of WNK3 (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-39258A, sc-39258B and sc-39258C.

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

WNK3 siRNA (h) is recommended for the inhibition of WNK3 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

WNK3 (A-6): sc-515570 is recommended as a control antibody for monitoring of WNK3 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 MarkerTM 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 WNK3 gene expression knockdown using RT-PCR Primer: WNK3 (h)-PR: sc-39258-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ 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.