

ANKRD44 siRNA (m): sc-141103

BACKGROUND

Ankyrins are membrane adaptor molecules that play important roles in coupling integral membrane proteins to the spectrin-based cytoskeleton network. Mutations of ankyrin genes lead to severe genetic diseases, such as fatal cardiac arrhythmias and hereditary spherocytosis. ANKRD44 (ankyrin repeat domain 44), also known as PP6-ARS-B (serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit B), is a 993 amino acid protein that contains 28 ANK repeats and exists as 5 alternatively spliced isoforms. Encoded by a gene that maps to human chromosome 2q33.1, ANKRD44 is conserved in chimpanzee, canine, mouse, rat, chicken, zebrafish and *Magnaporthe grisea*. ANKRD44 is a putative regulatory subunit of protein phosphatase 6 (PP6), a holoenzyme that may be a heterotrimeric complex formed by a catalytic subunit, a SKAP55 domain-containing subunit (PP6R) and an ankyrin repeat-domain containing regulatory subunit (ARS). ANKRD44 may also be involved in phosphoprotein substrate recognition.

REFERENCES

- Altman, A.L. and Fanning, E. 2001. The Chinese hamster dihydrofolate reductase replication origin beta is active at multiple ectopic chromosomal locations and requires specific DNA sequence elements for activity. *Mol. Cell. Biol.* 21: 1098-1110.
- Ota, T., et al. 2004. Complete sequencing and characterization of 21,243 full-length human cDNAs. *Nat. Genet.* 36: 40-45.
- Bechtel, S., et al. 2007. The full-ORF clone resource of the german cDNA consortium. *BMC Genomics* 8: 399.
- Stefansson, B., et al. 2008. Protein phosphatase 6 regulatory subunits composed of ankyrin repeat domains. *Biochemistry* 47: 1442-1451.
- Guerignon, J., et al. 2009. Mapping of protein phosphatase-6 association with its SAPS domain regulatory subunit using a model of helical repeats. *BMC Biochem.* 10: 24.
- Douglas, P., et al. 2010. Protein phosphatase 6 interacts with the DNA-dependent protein kinase catalytic subunit and dephosphorylates γ -H2AX. *Mol. Cell. Biol.* 30: 1368-1381.

CHROMOSOMAL LOCATION

Genetic locus: Ankrd44 (mouse) mapping to 1 C1.1.

PRODUCT

ANKRD44 siRNA (m) is a pool of 2 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 ANKRD44 shRNA Plasmid (m): sc-141103-SH and ANKRD44 shRNA (m) Lentiviral Particles: sc-141103-V as alternate gene silencing products.

For independent verification of ANKRD44 (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-141103A and sc-141103B.

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

ANKRD44 siRNA (m) is recommended for the inhibition of ANKRD44 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

ANKRD28/44/52 (G-6): sc-393032 is recommended as a control antibody for monitoring of ANKRD44 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 ANKRD44 gene expression knockdown using RT-PCR Primer: ANKRD44 (m)-PR: sc-141103-PR (20 μ 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.