

UBXD4 siRNA (h): sc-94677

BACKGROUND

The UBX (ubiquitin regulatory X) domain is an 80 amino acid motif that is usually present on the carboxy-terminus of certain eukaryotic proteins. UBX domain-containing proteins (UBXD), such as FAF1, p33ING1 and D8S2298E, are typically involved in ubiquitin-related processes. UBXD proteins also constitute the largest family of VCP cofactors and are generally involved in substrate recruitment to VCP, as well as regulation of its activity. UBXD4 (UBX domain-containing protein 4), also known as UBXN2A (UBX domain-containing protein 2A), is a 259 amino acid protein that contains one UBX domain and is located in both *cis*-Golgi and ER compartments. Overexpression of UBXD4 in differentiated PC12 cells leads to increased neuronal nicotinic acetylcholine receptor expression, especially of the $\alpha 3\beta 2$ subtype, on the cell surface. UBXD4 can also interfere with the ubiquitination of the $\alpha 3$ subunit, leading to its stabilization and, consequently, the stabilization of cell surface receptors.

REFERENCES

1. Buchberger, A. 2002. From UBA to UBX: new words in the ubiquitin vocabulary. *Trends Cell Biol.* 12: 216-221.
2. Dreveny, I., et al. 2004. Structural basis of the interaction between the AAA ATPase p97/VCP and its adaptor protein p47. *EMBO J.* 23: 1030-1039.
3. Bruderer, R.M., et al. 2004. The AAA ATPase p97/VCP interacts with its alternative co-factors, Ufd1-Npl4 and p47, through a common bipartite binding mechanism. *J. Biol. Chem.* 279: 49609-49616.
4. Yeung, H.O., et al. 2008. Insights into adaptor binding to the AAA protein p97. *Biochem. Soc. Trans.* 36: 62-67.
5. Schubert, C., et al. 2008. UBX domain proteins: major regulators of the AAA ATPase Cdc48/p97. *Cell. Mol. Life Sci.* 65: 2360-2371.
6. Kern, M., et al. 2009. UBXD1 binds p97 through two independent binding sites. *Biochem. Biophys. Res. Commun.* 380: 303-307.
7. Nagahama, M., et al. 2009. UBXD1 is a VCP-interacting protein that is involved in ER-associated degradation. *Biochem. Biophys. Res. Commun.* 382: 303-308.
8. Rezvani, K., et al. 2009. UBXD4, a UBX-containing protein, regulates the cell surface number and stability of $\alpha 3$ -containing nicotinic acetylcholine receptors. *J. Neurosci.* 29: 6883-6896.

CHROMOSOMAL LOCATION

Genetic locus: UBXN2A (human) mapping to 2p23.3.

PRODUCT

UBXD4 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 UBXD4 shRNA Plasmid (h): sc-94677-SH and UBXD4 shRNA (h) Lentiviral Particles: sc-94677-V as alternate gene silencing products.

For independent verification of UBXD4 (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-94677A, sc-94677B and sc-94677C.

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

UBXD4 siRNA (h) is recommended for the inhibition of UBXD4 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor UBXD4 gene expression knockdown using RT-PCR Primer: UBXD4 (h)-PR: sc-94677-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.