

FBXO4 siRNA (h): sc-91910

BACKGROUND

F-box proteins are critical components of the SCF (Skp1-CUL-1-F-box protein)-type E3 ubiquitin ligase complex and are involved in substrate recognition and recruitment for ubiquitination. F-box proteins are members of a large family that regulates cell cycle, immune response, signalling cascades and developmental programs by targeting proteins, such as cyclins, cyclin-dependent kinase inhibitors, I κ B- α and β -catenin, for degradation by the proteasome after ubiquitination. F-box only protein 4 (FBXO4) is a substrate recognition component of the SCF-type E3 ubiquitin ligase complex, possibly involved in the recognition and binding to phosphorylated target proteins. FBXO4 directly interacts with Skp1 p19 and CUL-1 within the SCF-type E3 complex and has been found to recognize TRF1 and promote its ubiquitination. FBXO4 is expressed as two isoforms produced by alternative splicing.

REFERENCES

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2. Winston, J.T., et al. 1999. A family of mammalian F-box proteins. *Curr. Biol.* 9: 1180-1182.
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4. Jin, J., et al. 2004. Systematic analysis and nomenclature of mammalian F-box proteins. *Genes Dev.* 18: 2573-2580.
5. Lee, T.H., et al. 2006. The F-box protein FBX4 targets PIN2/TRF1 for ubiquitin-mediated degradation and regulates telomere maintenance. *J. Biol. Chem.* 281: 759-768.
6. Lin, D.I., et al. 2006. Phosphorylation-dependent ubiquitination of cyclin D1 by the SCF(FBX4- α B crystallin) complex. *Mol. Cell* 24: 355-366.
7. Liu, Y., et al. 2006. The ETS protein MEF is regulated by phosphorylation-dependent proteolysis via the protein-ubiquitin ligase SCFSkp2. *Mol. Cell Biol.* 26: 3114-3123.
8. Barbash, O., et al. 2008. Mutations in Fbx4 inhibit dimerization of the SCF(Fbx4) ligase and contribute to cyclin D1 overexpression in human cancer. *Cancer Cell* 14: 68-78.

CHROMOSOMAL LOCATION

Genetic locus: FBXO4 (human) mapping to 5p13.1.

PRODUCT

FBXO4 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 FBXO4 shRNA Plasmid (h): sc-91910-SH and FBXO4 shRNA (h) Lentiviral Particles: sc-91910-V as alternate gene silencing products.

For independent verification of FBXO4 (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-91910A, sc-91910B and sc-91910C.

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

FBXO4 siRNA (h) is recommended for the inhibition of FBXO4 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

FBXO4 (D-9): sc-376372 is recommended as a control antibody for monitoring of FBXO4 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 FBXO4 gene expression knockdown using RT-PCR Primer: FBXO4 (h)-PR: sc-91910-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.