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# Smurf1 siRNA (h): sc-41673

## BACKGROUND

Smurf1 and Smurf2 (Smad ubiquitination regulatory factor-1 and 2) are members of the Hect family of proteins, which also includes the ubiquitin (Ub) E3-type ligases Nedd3 and E6-AP. E3 ligases are involved in the enzymatic reactions of the Ub conjugating pathway, which targets proteins for degradation by the 26S Proteasome. Within the Ub pathway, the E3 ligases specifically catalyze the transfer of Ub from the Ub-conjugating enzymes to the individual protein substrate. As an E3 ligase, Smurf1 selectively interacts with receptor-regulated SMADs specific to the BMP pathway in order to trigger their ubiquitination and degradation. Smurf2 interacts with receptor-activated Smads (R-Smads), including Smad1, Smad2, and Smad3, but not Smad4. Although Smurf2 localizes to the nucleus, binding to Smad7 induces its export and its recruitment to the activated TGF $\beta$  receptor, where it causes degradation of Smad7.

## REFERENCES

1. Scheffner, M., et al. 1993. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. *Cell* 75: 495-505.
2. Huibregtse, J.M., et al. 1995. A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. *Proc. Natl. Acad. Sci. USA* 92: 2563-2567.
3. Hershko, A., et al. 1998. The ubiquitin system. *Annu. Rev. Biochem.* 67: 425-479.
4. Zhu, H., et al. 1999. A Smad ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. *Nature* 400: 687-693.
5. Lin, X., et al. 2000. Smurf2 is a ubiquitin E3 ligase mediating proteasome-dependent degradation of Smad2 in transforming growth factor- $\beta$  signaling. *J. Biol. Chem.* 275: 36818-36822.

## CHROMOSOMAL LOCATION

Genetic locus: SMURF1 (human) mapping to 7q22.1.

## PRODUCT

Smurf1 siRNA (h) is a target-specific 19-25 nt siRNA 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 Smurf1 shRNA Plasmid (h): sc-41673-SH and Smurf1 shRNA (h) Lentiviral Particles: sc-41673-V as alternate gene silencing products.

## STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$  C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$  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

Smurf1 siRNA (h) is recommended for the inhibition of Smurf1 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  $\mu$ 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

Smurf1 (C-11): sc-518118 is recommended as a control antibody for monitoring of Smurf1 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<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> 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<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Smurf1 gene expression knockdown using RT-PCR Primer: Smurf1 (h)-PR: sc-41673-PR (20  $\mu$ l, 496 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$  C and the extension temperature should be 68-72 $^{\circ}$  C.

## SELECT PRODUCT CITATIONS

1. Shukla, S., et al. 2014. KRAS protein stability is regulated through Smurf2: UBCH5 complex-mediated  $\beta$ -TrCP1 degradation. *Neoplasia* 16: 115-128.
2. Yu, L., et al. 2015. SND1 acts downstream of TGF $\beta$ 1 and upstream of Smurf1 to promote breast cancer metastasis. *Cancer Res.* 75: 1275-1286.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.