



SUG1 siRNA (h): sc-76603

BACKGROUND

The 26S Proteasome is a highly ordered proteinase complex consisting of a 20S core and a 19S regulator. While the core is responsible for the proteolytic activity of the proteasome, the regulator contains several ATPase subunits which function in the ATP-dependent degradation of ubiquitinated proteins and confer substrate specificity to the 26S complex. SUG1, also known as PSMC5 (Proteasome 26S subunit ATPase 5), p45 or S8, is an ATPase subunit that is an integral part of the 26S Proteasome complex. Localized to the cytoplasm and nucleus, SUG1 is part of the 19S regulator and functions in the ubiquitin/proteasome-mediated degradation of proteins (specifically receptors) found in the endoplasmic reticulum (ER). Recent studies suggest that assembly of the 26S Proteasome is dependent upon phosphorylation of SUG1 by a protein kinase. *In vitro*, SUG1 also interacts with RXR (retinoid X receptor) and TR (thyroid hormone receptor), suggesting a possible role in transcriptional regulation.

REFERENCES

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- Makino, Y., et al. 1997. SUG1, a component of the 26S Proteasome, is an ATPase stimulated by specific RNAs. *J. Biol. Chem.* 272: 23201-23205.
- Masuyama, H. and MacDonald, P.N. 1999. Proteasome-mediated degradation of the vitamin D receptor (VDR) and a putative role for SUG1 interaction with the AF-2 domain of VDR. *J. Cell. Biochem.* 71: 429-440.
- Su, K., et al. 2000. Human SUG1/p45 is involved in the proteasome-dependent degradation of Sp1. *Biochem. J.* 348: 281-289.
- Chang, C., et al. 2001. The GAL4 activation domain binds SUG2 protein, a proteasome component, *in vivo* and *in vitro*. *J. Biol. Chem.* 276: 30956-30963.
- Gianni, M., et al. 2002. Phosphorylation by p38 MAPK and recruitment of SUG1 are required for RA-induced RAR γ degradation and transactivation. *EMBO J.* 21: 3760-3769.

CHROMOSOMAL LOCATION

Genetic locus: PSMC5 (human) mapping to 17q23.3.

PRODUCT

SUG1 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 SUG1 shRNA Plasmid (h): sc-76603-SH and SUG1 shRNA (h) Lentiviral Particles: sc-76603-V as alternate gene silencing products.

For independent verification of SUG1 (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-76603A, sc-76603B and sc-76603C.

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

SUG1 siRNA (h) is recommended for the inhibition of SUG1 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

SUG1 (H-7): sc-390631 is recommended as a control antibody for monitoring of SUG1 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 SUG1 gene expression knockdown using RT-PCR Primer: SUG1 (h)-PR: sc-76603-PR (20 μ l, 567 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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