SANTA CRUZ BIOTECHNOLOGY, INC.

p44S10 siRNA (m): sc-41384

BACKGROUND

In eukaryotic cells, selective breakdown of cellular proteins is ensured by two distinct pathways, ubiquitination and degradation by the 26S proteasome. At specific stages of development, embryo- and tissue-specific components of the 26S proteasome are formed by developmentally regulated alternative splicing, including Rpn10a through Rpn10e (also designated pUb-R2 through pUb-R5). The pUb-R2 subunit, originally identified as S5a, is ubiquitously expressed and may perform proteolysis constitutively in a wide variety of cells. p44S10 is a highly conserved proteasome regulatory subunit that is expressed in heart, liver, skeletal muscle and pancreas. In addition to normal tissue expression, p44S10 is also expressed in several melanoma cell lines, such as MCF-7, 451Lu and WM164. Since forced expression of p44S10 in radial growth phase melanoma cells results in an increase in cellular proliferation, p44S10 may represent a potential link between regulation of proteasome activity and tumor cell proliferation *in vivo*.

REFERENCES

1. Lonnroth, I. and Lange, S. 1986. Purification and characterization of the antisecretory factor: a protein in the central nervous system and in the gut which inhibits intestinal hypersecretion induced by cholera toxin. Biochim. Biophys. Acta 883: 138-144.
2. Johansson, E., et al. 1995. Molecular cloning and expression of a pituitary gland protein modulating intestinal fluid secretion. J. Biol. Chem. 270: 20615-20620.
3. Coux, O., et al. 1996. Structure and functions of the 20S and 26S Proteasomes. Annu. Rev. Biochem. 65: 801-847.
4. Voges, D., et al. 1999. The 26S Proteasome: a molecular machine designed for controlled proteolysis. Annu. Rev. Biochem. 68: 1015-1068.
5. Ren, S., et al. 2000. The p44S10 locus, encoding a subunit of the proteasome regulatory particle, is amplified during progression of cutaneous malignant melanoma. Oncogene 19: 1419-1427.
6. Kawahara, H., et al. 2000. Developmentally regulated, alternative splicing of the Rpn10 gene generates multiple forms of 26S proteasomes. EMBO J. 19: 4144-4153.

CHROMOSOMAL LOCATION

Genetic locus: Psmd6 (mouse) mapping to 14 A1.

PRODUCT

p44S10 siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see p44S10 shRNA Plasmid (m): sc-41384-SH and p44S10 shRNA (m) Lentiviral Particles: sc-41384-V as alternate gene silencing products.

For independent verification of p44S10 (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-41384A, sc-41384B and sc-41384C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

p44S10 siRNA (m) is recommended for the inhibition of p44S10 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 µM in 66 µl. 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

p44S10 (E-12): sc-393580 is recommended as a control antibody for monitoring of p44S10 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 p44S10 gene expression knockdown using RT-PCR Primer: p44S10 (m)-PR: sc-41384-PR (20 µl). 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.