SANTA CRUZ BIOTECHNOLOGY, INC.

20S Proteasome α1 siRNA (h): sc-45256

BACKGROUND

Ubiquitin-dependent proteolysis mediates selective destruction of various cell cycle regulators, transcription factors and tumor suppressors. In eukaryotic cells, selective breakdown of cellular proteins is ensured by their ubiquitination and subsequent degradation by the 26S proteasome. At specific stages of development, embryo- and tissue-specific components of the 26S proteasome form, facilitating proteolysis. 20S Proteasome α1, also designated macropain subunit C2 or PROS-30, is a prosomal protein involved in a non-lysosomal ATP/ubiquitin-dependent proteolytic pathway. The entire proteasome is composed of at least 15 non-identical subunits which form a highly-ordered ring-shaped structure.

REFERENCES

1. Silva Pereira, I., et al. 1992. Two mRNAs exist for the Hs PROS-30 gene encoding a component of human prosomes. Gene 120: 235-242.
2. Bey, F., et al. 1993. The prosomal RNA-binding protein p27K is a member of the α-type human prosomal gene family. Mol. Gen. Genet. 237: 193-205.
3. Kristensen, P., et al. 1994. Human proteasome subunits from 2-dimensional gels identified by partial sequencing. Biochem. Biophys. Res. Commun. 205: 1785-1789.
4. Zaiss, D. and Belote, J.M. 1997. Molecular cloning of the *Drosophila melanogaster* gene α5_dm encoding a 20S Proteasome α-type subunit. Gene 201: 99-105.
5. Knipfer, N., et al. 1999. Species variation in ATP-dependent protein degradation: protease profiles differ between mycobacteria and protease functions differ between *Mycobacterium smegmatis* and *Escherichia coli*. Gene 231: 95-104.
6. Whitehouse, A.S., et al. 2001. Mechanism of attenuation of skeletal muscle protein catabolism in cancer cachexia by eicosapentaenoic acid. Cancer Res. 61: 3604-3609.
7. Touitou, R., et al. 2001. A degradation signal located in the C-terminus of $p21^{WAF1/CIP1}$ is a binding site for the C8α-subunit of the 20S proteasome. EMBO J. 20: 2367-2375.

CHROMOSOMAL LOCATION

Genetic locus: PSMA1 (human) mapping to 11p15.2.

PRODUCT

20S Proteasome α1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see 20S Proteasome α1 shRNA Plasmid (h): sc-45256-SH and 20S Proteasome α1 shRNA (h) Lentiviral Particles: sc-45256-V as alternate gene silencing products.

For independent verification of 20S Proteasome α1 (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-45256A, sc-45256B and sc-45256C.

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

20S Proteasome α1 siRNA (h) is recommended for the inhibition of 20S Proteasome α1 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 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

20S Proteasome α1 (C-7): sc-166073 is recommended as a control antibody for monitoring of 20S Proteasome α1 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 20S Proteasome α1 gene expression knockdown using RT-PCR Primer: 20S Proteasome α1 (h)-PR: sc-45256-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.