

20S Proteasome β 5 siRNA (h): sc-62872

BACKGROUND

The proteasome represents a large protein complex that exists inside all eukaryotes and archaea, and in some bacteria. The main function of proteasomes is to degrade unnecessary or damaged proteins by proteolysis. The most common form of the proteasome, known as the 26S Proteasome, contains one 20S Proteasome core particle structure and two 19S regulatory caps. The 20S Proteasome core is hollow and forms an enclosed cavity, where proteins are degraded, as well as openings at the two ends to allow the target protein to enter. The 20S Proteasome core particle contains many subunits, depending on the organism. All of the subunits fall into one of two types: α subunits, which are structural, serve as docking domains for the regulatory particles and exterior gates blocking unregulated access to the interior cavity; or β subunits, which are predominantly catalytic. The outer two rings in the proteasome consist of seven α subunits each, and the inner two rings each consist of seven β subunits.

REFERENCES

1. Kristensen, P., et al. 1995. Human proteasome subunits from two-dimensional gels identified by partial sequencing. *Biochem. Biophys. Res. Commun.* 205: 1785-1789.
2. Morimoto, Y., et al. 1995. Ordered structure of the crystallized bovine 20S Proteasome. *J. Biochem.* 117: 471-474.
3. Wenzel, T., et al. 1995. Conformational constraints in protein degradation by the 20S Proteasome. *Nat. Struct. Biol.* 2: 199-204.
4. Schmidt, M., et al. 1997. Structure and structure formation of the 20S Proteasome. *Mol. Biol. Rep.* 24: 103-112.

CHROMOSOMAL LOCATION

Genetic locus: PSMB5 (human) mapping to 14q11.2.

PRODUCT

20S Proteasome β 5 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 20S Proteasome β 5 shRNA Plasmid (h): sc-62872-SH and 20S Proteasome 5 shRNA (h) Lentiviral Particles: sc-62872-V as alternate gene silencing products.

For independent verification of 20S Proteasome β 5 (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-62872A, sc-62872B and sc-62872C.

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

20S Proteasome β 5 siRNA (h) is recommended for the inhibition of 20S Proteasome β 5 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

20S Proteasome β 5 (A-10): sc-393931 is recommended as a control antibody for monitoring of 20S Proteasome β 5 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 20S Proteasome β 5 gene expression knockdown using RT-PCR Primer: 20S Proteasome β 5 (h)-PR: sc-62872-PR (20 μ l, 574 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. Asaka, M., et al. 2011. Rab5a-mediated localization of claudin-1 is regulated by proteasomes in endothelial cells. *Am. J. Physiol., Cell Physiol.* 300: C87-C96.

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.