

## 20S Proteasome $\beta$ 3 siRNA (h): sc-62868

### 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:  $\alpha$  subunits, which are structural, serve as docking domains for the regulatory particles and exterior gates blocking unregulated access to the interior cavity; or  $\beta$  subunits, which are predominantly catalytic. The outer two rings in the proteasome consist of seven  $\alpha$  subunits each, and the inner two rings each consist of seven  $\beta$  subunits.

### REFERENCES

- Kristensen, P., Johnsen, A.H., Uerkvitz, W., Tanaka, K. and Hendil, K.B. 1995. Human proteasome subunits from two-dimensional gels identified by partial sequencing. *Biochem. Biophys. Res. Commun.* 205: 1785-1789.
- Morimoto, Y., Mizushima, T., Yagi, A., Tanahashi, N., Tanaka, K., Ichihara, A. and Tsukihara, T. 1995. Ordered structure of the crystallized bovine 20S Proteasome. *J. Biochem.* 117: 471-474.
- Wenzel, T. and Baumeister, W. 1995. Conformational constraints in protein degradation by the 20S Proteasome. *Nat. Struct. Biol.* 2: 199-204.
- Schmidt, M., Schmidtke, G. and Kloetzel, P.M. 1997. Structure and structure formation of the 20S Proteasome. *Mol. Biol. Rep.* 24: 103-112.
- Sassa, H., Oguchi, S., Inoue, T. and Hirano, H. 2000. Primary structural features of the 20S Proteasome subunits of rice (*Oryza sativa*). *Gene* 250: 61-66.
- Ferrington, D.A. and Kappahn, R.J. 2004. Catalytic site-specific inhibition of the 20S Proteasome by 4-hydroxynonenal. *FEBS Lett.* 578: 217-223.
- Huang, L. and Burlingame, A.L. 2006. Comprehensive mass spectrometric analysis of the 20S Proteasome complex. *Meth. Enzymol.* 405: 187-236.
- Madding, L.S., Michel, J.K., Shockley, K.R., Connors, S.B., Epting, K.L., Johnson, M.R. and Kelly, R.M. 2006. Role of the  $\beta$ 1 subunit in the function and stability of the 20S Proteasome in the hyperthermophilic archaeon *Pyrococcus furiosus*. *J. Bacteriol.* 189: 583-590.
- Ryzewski, R.M., Burrill, L., Mendonca, R., Palmer, J.T., Rice, M., Tahilramani, R., Bass, K.E., Leung, L., Gjerstad, E., Janc, J.W. and Pan, L. 2006. Optimization of subsite binding to the  $\beta$ 5 subunit of the human 20S Proteasome using vinyl sulfones and 2-keto-1,3,4-oxadiazoles: syntheses and cellular properties of potent, selective proteasome inhibitors. *J. Med. Chem.* 49: 2953-2968.

### PROTOCOLS

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

### CHROMOSOMAL LOCATION

Genetic locus: PSMB3 (human) mapping to 17q12.

### PRODUCT

20S Proteasome  $\beta$ 3 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see 20S Proteasome  $\beta$ 3 shRNA Plasmid (h): sc-62868-SH and 20S Proteasome  $\beta$ 3 shRNA (h) Lentiviral Particles: sc-62868-V as alternate gene silencing products.

For independent verification of 20S Proteasome  $\beta$ 3 (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-62868A, sc-62868B and sc-62868C.

### 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  $\mu$ 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

20S Proteasome  $\beta$ 3 siRNA (h) is recommended for the inhibition of 20S Proteasome  $\beta$ 3 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.

### RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor 20S Proteasome  $\beta$ 3 gene expression knockdown using RT-PCR Primer: 20S Proteasome  $\beta$ 3 (h)-PR: sc-62868-PR (20  $\mu$ l). 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.