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# 20S Proteasome $\alpha$ 1 shRNA (h) Lentiviral Particles: sc-45256-V

## 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  $\alpha$ 1, 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  $\alpha$ -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. 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.
5. 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.
6. Tuitou, R., et al. 2001. A degradation signal located in the C-terminus of p21WAF1/CIP1 is a binding site for the C8 $\alpha$ -subunit of the 20S proteasome. *EMBO J.* 20: 2367-2375.

## CHROMOSOMAL LOCATION

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

## PRODUCT

20S Proteasome  $\alpha$ 1 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see 20S Proteasome  $\alpha$ 1 siRNA (h): sc-45256 and 20S Proteasome  $\alpha$ 1 shRNA Plasmid (h): sc-45256-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ$  C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

20S Proteasome  $\alpha$ 1 shRNA (h) Lentiviral Particles is recommended for the inhibition of 20S Proteasome  $\alpha$ 1 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

20S Proteasome  $\alpha$ 1 (C-7): sc-166073 is recommended as a control antibody for monitoring of 20S Proteasome  $\alpha$ 1 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor 20S Proteasome  $\alpha$ 1 gene expression knockdown using RT-PCR Primer: 20S Proteasome  $\alpha$ 1 (h)-PR: sc-45256-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ$  C and the extension temperature should be  $68-72^\circ$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

## PROTOCOLS

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