SANTA CRUZ BIOTECHNOLOGY, INC.

LMP7A siRNA (h): sc-42898

BACKGROUND

The eukaryotic multicatalytic proteinase complex, otherwise known as the proteasome, is present in both the nucleus and cytoplasm of cells and contains at least 15 nonidentical subunits, which form a highly ordered RING-shaped structure. The proteasome is involved in an ATP/ubiquitin-dependent proteolytic pathway and expresses at least five distinct proteolytic activities, including the cleavage of peptides after branched chain amino acids or bulky hydrophobic amino acids. Two components of the proteasome are the low molecular mass proteins LMP2 and LMP7, which are thought to connect the proteasome to the MHC class-I antigen-processing pathway. Upon stimulation with IFN-γ, LMP2 and LMP7 displace housekeeping subunits in the proteasome and activate cytotoxic T cells (CTLs). LMP2 and LMP7 are produced as precursor proteins, which are processed to subunits that have the ability to complex with the proteasome. LMP2 is expressed as two alternatively spliced forms, LMP2.l and LMP2.s, in lymphoblastoid cell lines and in fibroblasts after IFN-γ stimulation. LMP7 is also expressed as two forms, LMP7-E1 and E2, in several tissues.

REFERENCES

1. Fruh, K., et al. 1992. Alternative exon usage and processing of the major histocompatibility complex-encoded proteasome subunits. J. Biol. Chem. 267: 22131-22140.
2. Glynne, R., et al. 1993. The major histocompatibility complex-encoded proteasome component LMP7: alternative first exons and post-translational processing. Eur. J. Immunol. 23: 860-866.
3. Cardozo, C. 1993. Catalytic components of the bovine pituitary multicatalytic proteinase complex (proteasome). Enzyme Protein 47: 296-305.
4. Frentzel, S., et al. 1993. The major-histocompatibility-complex-encoded β-type proteasome subunits LMP2 and LMP7. Evidence that LMP2 and LMP7 are synthesized as proproteins and that cellular levels of both mRNA and LMP-containing 20S proteasomes are differentially regulated. Eur. J. Biochem. 216: 119-126.
5. Figueiredo-Pereira, M.E., et al. 1994. Dissociation and reassociation of the bovine pituitary multicatalytic proteinase complex. J. Biol. Chem. 269: 621-666.

CHROMOSOMAL LOCATION

Genetic locus: PSMB8 (human) mapping to 6p21.32.

PRODUCT

LMP7A 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 LMP7A shRNA Plasmid (h): sc-42898-SH and LMP7A shRNA (h) Lentiviral Particles: sc-42898-V as alternate gene silencing products.

For independent verification of LMP7A (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-42898A, sc-42898B and sc-42898C.

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

LMP7A siRNA (h) is recommended for the inhibition of LMP7A 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

LMP7 (A-12): sc-365699 is recommended as a control antibody for monitoring of LMP7A 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 LMP7A gene expression knockdown using RT-PCR Primer: LMP7A (h)-PR: sc-42898-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.