SANTA CRUZ BIOTECHNOLOGY, INC.

PA28β siRNA (h): sc-40798

BACKGROUND

PA28 is an interferon-γ (IFN-γ) inducible proteasome activator required for presentation of certain major histocom-patibility (MHC) class I antigens. The PA28 complex is composed of two homologous subunits, α and β, which have similar catalytic properties and associate to form a hexameric ring. PA28α and PA28β, form a heteropolymer that binds to both ends of the 20S proteasome. In the mouse genome, two different chromosomal loci exist for PA28β, both of which are transcribed and encode a functional PA28β subunit. PA28β, for Proteasome activator 28β, is also known as PSME2, REG-β and proteasome (prosome, macropain) activator subunit 2. PA28β is a strong proteasome activator, although its affinity for the proteasome is about 10-fold less than recombinant PA28α. The PA28 complex is expressed constitutively in antigen-presenting cells. Downregulation of PA28 results in abnormal proteasome activation and has been implicated in the development of intimal hyperplasia (IH) in animal models.

REFERENCES

1. Kohda, K., et al. 1998. Characterization of the mouse PA28 activator complex gene family: complete organizations of the three member genes and a physical map of the approximately 150-kb region containing the α- and β-subunit genes. J. Immunol. 160: 4923-4935.
2. Zaiss, D.M. and Kloetzel, P.M. 1999. A second gene encoding the mouse proteasome activator PA28β subunit is part of a LINE1 element and is driven by a LINE1 promoter. J. Mol. Biol. 287: 829-835.
3. Wilk, S., et al. 2000. Properties of the β subunit of the proteasome activator PA28 (11S REG). Arch. Biochem. Biophys. 384: 74-180.
4. Fabunmi, R.P., et al. 2001. Interferon-γ regulates accumulation of the proteasome activator PA28 and immunoproteasomes at nuclear PML bodies. J. Cell Sci. 114: 29-36.
5. Faries, P.L., et al. 2001. Relationship of the 20S proteasome and the pro-teasome activator PA28 to atherosclerosis and intimal hyperplasia in the human vascular system. Ann. Vasc. Surg. 15: 628-633.
6. Murata, S., et al. 2001. Immunopro-teasome assembly and antigen presen-tation in mice lacking both PA28α and PA28β. EMBO J. 20: 5898-5907.

CHROMOSOMAL LOCATION

Genetic locus: PSME2 (human) mapping to 14q12.

PRODUCT

PA28β 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 PA28β shRNA Plasmid (h): sc-40798-SH and PA28β shRNA (h) Lentiviral Particles: sc-40798-V as alter-nate gene silencing products.

For independent verification of PA28β (h) gene silencing results, we also pro-vide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-40798A, sc-40798B and sc-40798C.

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

PA28β siRNA (h) is recommended for the inhibition of PA28β 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 recom-mended. 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

PA28β (G-10): sc-390563 is recommended as a control antibody for monitor-ing of PA28β 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) Immunofluo-rescence: 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 PA28β gene expres-sion knockdown using RT-PCR Primer: PA28β (h)-PR: sc-40798-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.