SANTA CRUZ BIOTECHNOLOGY, INC.

PA28α siRNA (m): sc-151977

BACKGROUND

PA28 is an interferong (IFNγ) 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. The PMSE2 gene maps to chromosome 14q11.2 and encodes the β subunit of the proteasome activator PA28.

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. Wilk, S., et al. 2000. Properties of the β subunit of the proteasome activator PA28 (11S REG). Arch. Biochem. Biophys. 384: 174-180.
3. 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.

CHROMOSOMAL LOCATION

Genetic locus: Psme1 (mouse) mapping to 14 C3.

PRODUCT

PA28α siRNA (m) 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 PA28α shRNA Plasmid (m): sc-151977-SH and PA28α shRNA (m) Lentiviral Particles: sc-151977-V as alternate gene silencing products.

For independent verification of PA28α (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-151977A, sc-151977B and sc-151977C.

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

PA28α siRNA (m) is recommended for the inhibition of PA28α expression in mouse 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

PA28α (G-9): sc-518190 is recommended as a control antibody for monitoring of PA28α 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 PA28α gene expression knockdown using RT-PCR Primer: PA28α (m)-PR: sc-151977-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Pickering, A.M., et al. 2010. The immunoproteasome, the 20S Proteasome and the PA28αβ proteasome regulator are oxidative-stress-adaptive proteolytic complexes. Biochem. J. 432: 585-594.
2. Pickering, A.M., et al. 2012. Nrf2-dependent induction of proteasome and PA28αβ regulator are required for adaptation to oxidative stress. J. Biol. Chem. 287: 10021-10031.
3. Pickering, A.M. and Davies, K.J. 2012. Differential roles of proteasome and immunoproteasome regulators PA28αβ, PA28γ and PA200 in the degradation of oxidized proteins. Arch. Biochem. Biophys. 523: 181-190.

RESEARCH USE

For research use only, not for use in diagnostic procedures.