SANTA CRUZ BIOTECHNOLOGY, INC.

PRAT4A siRNA (m): sc-152441

The Power to Question

BACKGROUND

Toll-like receptors (TLRs) are responsible for the innate recognition of microbial products and the induction of immune responses. There are two types of TLRs, cell surface and intracellular, but both rely on their subcellular distribution for optimal microbial recognition. TLR4, a cell surface TLR, is a member of the Toll family that detects lipopolysaccharide (LPS), which is an endotoxin that activates immune cells such as macrophages and dendritic cells. LPS recognition by TLR4 is induced by MD-2, an extracellular molecule that binds to the extracellular domain of TLR4, and PRAT4A (protein associated with toll-Like receptor 4), which regulates the cell surface expression of TLR4. PRAT4A, which is also known as CAG4A or TNRC5, is a 278 amino acid protein and is expressed as 2 isoforms due to alternative splicing events.

REFERENCES

1. Muzio, M., et al. 2000. Toll-like receptor family and signalling pathway. Biochem. Soc. Trans. 28: 563-566.
2. Akashi, S., et al. 2000. Cutting edge: cell surface expression and lipopolysaccharide signaling via the toll-like receptor 4-MD-2 complex on mouse peritoneal macrophages. J. Immunol. 164: 3471-3475.
3. Kirschning, C.J. and Schumann, R.R. 2002. TLR2: cellular sensor for microbial and endogenous molecular patterns. Curr. Top. Microbiol. Immunol. 270: 121-144.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 610047. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Kawasaki, K., et al. 2003. Identification of mouse MD-2 residues important for forming the cell surface TLR4-MD-2 complex recognized by anti-TLR4-MD-2 antibodies, and for conferring LPS and taxol responsiveness on mouse TLR4 by alanine-scanning mutagenesis. J. Immunol. 170: 413-420.
6. Konno, K., et al. 2006. A molecule that is associated with Toll-like receptor 4 and regulates its cell surface expression. Biochem. Biophys. Res. Commun. 339: 1076-1082.
7. Saitoh, S. and Miyake, K. 2006. Mechanism regulating cell surface expression and activation of Toll-like receptor 4. Chem. Rec. 6: 311-319.

CHROMOSOMAL LOCATION

Genetic locus: Cnpy3 (mouse) mapping to 17 C.

PRODUCT

PRAT4A 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 PRAT4A shRNA Plasmid (m): sc-152441-SH and PRAT4A shRNA (m) Lentiviral Particles: sc-152441-V as alternate gene silencing products.

For independent verification of PRAT4A (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-152441A, sc-152441B and sc-152441C.

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

PRAT4A siRNA (m) is recommended for the inhibition of PRAT4A 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

PRAT4A (F-6): sc-515151 is recommended as a control antibody for monitoring of PRAT4A 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 PRAT4A gene expression knockdown using RT-PCR Primer: PRAT4A (m)-PR: sc-152441-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.