

PRAT4B siRNA (h): sc-89627

BACKGROUND

PRAT4B, also known as CNPY4 (canopy 4 homolog), is a 248 amino acid protein that contains one saposin B-type domain and belongs to the canopy family. Localizing to endoplasmic reticulum, PRAT4B is ubiquitously expressed, with highest expression in lung, spleen, thymus and uterus. PRAT4B expression is linked to a decrease in cell surface TLR4, although the total cellular TLR4 protein level remains unchanged, indicating that PRAT4B is involved in the regulation of cell surface expression of TLR4. PRAT4B associates with TLR4 alone or with TLR4/MD2, but does not associate with MD2 alone or with TLR2, another Toll-like receptor. Expression of PRAT4B significantly increases in sepsis and severe sepsis patients, with highest levels in severe sepsis patients, suggesting that PRAT4B may play an important role in production and development of abnormal inflammatory response in children with sepsis. The gene that encodes PRAT4B maps to human chromosome 7q22.1.

REFERENCES

- Garson, J.A., et al. 1991. Improvement of HCV genome detection with "short" PCR products. *Lancet* 338: 1466-1467.
- Ketheesan, N., et al. 2002. Demonstration of a cell-mediated immune response in melioidosis. *J. Infect. Dis.* 186: 286-289.
- 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.
- Wakabayashi, Y., et al. 2006. A protein associated with Toll-like receptor 4 (PRAT4A) regulates cell surface expression of TLR4. *J. Immunol.* 177: 1772-1779.
- Takahashi, K., et al. 2007. A protein associated with Toll-like receptor (TLR) 4 (PRAT4A) is required for TLR-dependent immune responses. *J. Exp. Med.* 204: 2963-2976.
- Yang, W.G., et al. 2008. Role of transduce molecules and modulatory factors of signal pathways of Toll-like receptor in inflammatory response of children with sepsis. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue* 20: 561-564.
- Wang, G.B., et al. 2008. Changes and significance for regulatory factors for signal pathways of Toll-like receptors in immunological pathogenesis of Kawasaki disease. *Zhonghua Er Ke Za Zhi* 46: 49-54.

CHROMOSOMAL LOCATION

Genetic locus: CNPY4 (human) mapping to 7q22.1.

PRODUCT

PRAT4B 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PRAT4B shRNA Plasmid (h): sc-89627-SH and PRAT4B shRNA (h) Lentiviral Particles: sc-89627-V as alternate gene silencing products.

For independent verification of PRAT4B (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-89627A, sc-89627B and sc-89627C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PRAT4B siRNA (h) is recommended for the inhibition of PRAT4B 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 μ M in 66 μ l. 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

PRAT4B (E-10): sc-514307 is recommended as a control antibody for monitoring of PRAT4B 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 PRAT4B gene expression knockdown using RT-PCR Primer: PRAT4B (h)-PR: sc-89627-PR (20 μ l). 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.