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IRAK-4 siRNA (h): sc-45400

BACKGROUND

Interleukin-1 receptor (IL1R)-associated kinases (IRAKs) are important mediators in the signal transduction of Toll-like receptor (TLR) and IL1R family members, collectively referred to as TIRs. IRAK family members include two active kinases, IRAK-1 and IRAK-4, and two inactive kinases, IRAK-2 and IRAK-M. Binding of IL-1 to its cognate receptor results in the activation of the NF κ B signaling pathway and MAP kinase pathways. IRAK-4 appears to act upstream of other IRAKs and phosphorylates IRAK-1 on Threonine 387. It is highly expressed in liver and kidney tissues, but also displays a wide, low level of expression in other tissues. IRAK-4 is an essential component of innate immunity. Deficiency of IRAK-4 leads to recurrent bacterial infections and profound hyporesponsiveness to LPS and IL-1. Therefore, IRAK-4 may be a potential target for therapeutic drug design.

REFERENCES

- Li, S., et al. 2002. IRAK-4: a novel member of the IRAK family with the properties of an IRAK-kinase. *Proc. Natl. Acad. Sci. USA* 99: 5567-5572.
- Janssens, S., et al. 2003. Functional diversity and regulation of different interleukin-1 receptor-associated kinase (IRAK) family members. *Mol. Cell* 11: 293-302.
- Lye, E., et al. 2004. The role of interleukin 1 receptor-associated kinase-4 (IRAK-4) kinase activity in IRAK-4-mediated signaling. *J. Biol. Chem.* 279: 40653-40658.
- Medvedev, A.E., et al. 2005. Cutting edge: expression of IL-1 receptor-associated kinase-4 (IRAK-4) proteins with mutations identified in a patient with recurrent bacterial infections alters normal IRAK-4 interaction with components of the IL-1 receptor complex. *J. Immunol.* 174: 6587-6591.
- Lasker, M.V., et al. 2005. Cutting edge: molecular structure of the IL-1R-associated kinase-4 death domain and its implications for TLR signaling. *J. Immunol.* 175: 4175-4179.

CHROMOSOMAL LOCATION

Genetic locus: IRAK4 (human) mapping to 12q12.

PRODUCT

IRAK-4 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 IRAK-4 shRNA Plasmid (h): sc-45400-SH and IRAK-4 shRNA (h) Lentiviral Particles: sc-45400-V as alternate gene silencing products.

For independent verification of IRAK-4 (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-45400A, sc-45400B and sc-45400C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

IRAK-4 siRNA (h) is recommended for the inhibition of IRAK-4 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

IRAK-4 (G-2): sc-374349 is recommended as a control antibody for monitoring of IRAK-4 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 IRAK-4 gene expression knockdown using RT-PCR Primer: IRAK-4 (h)-PR: sc-45400-PR (20 μ l, 484 bp). 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.