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IRAK-4 shRNA (h) Lentiviral Particles: sc-45400-V

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 kinase, IRAK-2 and IRAK-M. Binding of IL-1 to its cognate receptor results in the activation of the NFκB 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

1. 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.
2. Janssens, S., et al. 2003. Functional diversity and regulation of different interleukin-1 receptor-associated kinase (IRAK) family members. *Mol. Cell* 11: 293-302.
3. 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.
4. 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. *J. Immunol.* 174: 6587-6591.
5. 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 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μl frozen stock containing 1.0 x 10⁶ lentiviral transducing particles per milliliter in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see IRAK-4 siRNA (h): sc-45400 and IRAK-4 shRNA Plasmid (h): sc-45400-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

IRAK-4 shRNA (h) Lentiviral Particles is recommended for the inhibition of IRAK-4 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μl frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 μl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.