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TIRAP siRNA (h): sc-42932

BACKGROUND

Mammalian Toll-like receptors (TLRs) recognize conserved products of microbial metabolism and activate NF κ B and other signaling pathways through the adapter protein MyD88. MyD88 functions as an adapter protein in the association of IL-1 receptor associated kinase (IRAK) with the IL-1 receptor. MyD88 contains a characteristic N-terminal death domain, which is essential for NF κ B activation, and an adjacent Toll/IL-1R homology domain (TIR domain), which is responsible for signal transduction. TIR domain-containing adapter protein (TIRAP), also designated MAL (MyD88 adapter-like), MyD88 or TLR-4 adaptor protein, is a cytoplasmic TIR-domain-containing protein that activates NF κ B, Jun N-terminal kinase and extracellular signal-regulated kinase-1 and -2. TIRAP forms homodimers and heterodimers with MyD88. IRAK-2, but not IRAK-1, is required for the activation of NF κ B by TIRAP which associates with IRAK-2 through the TIR domain. In addition, TIRAP associates with TLR-4, suggesting that it plays a role in TLR-4 signal transduction.

REFERENCES

1. Medzhitov, R., et al. 1998. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol. Cell* 2: 253-258.
2. Burns, K., et al. 1998. MyD88, an adaptor protein involved in interleukin-1 signaling. *J. Biol. Chem.* 273: 12203-12209.
3. Chow, J.C., et al. 1999. Toll-like receptor-4 mediates lipo-polysaccharide-induced signal transduction. *J. Biol. Chem.* 274: 10689-10692.
4. Means, T.K., et al. 2000. The biology of Toll-like receptors. *Cytokine Growth Factor Rev.* 11: 219-232.

CHROMOSOMAL LOCATION

Genetic locus: TIRAP (human) mapping to 11q24.2.

PRODUCT

TIRAP 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 TIRAP shRNA Plasmid (h): sc-42932-SH and TIRAP shRNA (h) Lentiviral Particles: sc-42932-V as alternate gene silencing products.

For independent verification of TIRAP (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-42932A, sc-42932B and sc-42932C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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

TIRAP siRNA (h) is recommended for the inhibition of TIRAP 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

TIRAP (A-11): sc-166149 is recommended as a control antibody for monitoring of TIRAP 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 MarkerTM 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 TIRAP gene expression knockdown using RT-PCR Primer: TIRAP (h)-PR: sc-42932-PR (20 μ l, 417 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.

SELECT PRODUCT CITATIONS

1. Hayes, T., et al. 2018. Innate immunity induced by the major allergen Alt a 1 from the fungus alternaria is dependent upon Toll-like receptors 2/4 in human lung epithelial cells. *Front. Immunol.* 9: 1507.

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.