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# TIRAP shRNA (m) Lentiviral Particles: sc-44740-V

## BACKGROUND

Mammalian Toll-like receptors (TLRs) recognize conserved products of microbial metabolism and activate NF $\kappa$ 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 $\kappa$ B activation, and an adjacent Toll/IL-1R homology domain (TIR domain), which is responsible for signal transduction. MAL (MyD88 adapter-like), also designated TIR domain-containing adapter protein (TIRAP), wyatt or TLR-4 adaptor protein, is a cytoplasmic TIR-domain-containing protein that activates NF $\kappa$ B, Jun amino-terminal kinase and extracellular signal-regulated kinase-1 and -2. MAL forms homodimers and heterodimers with MyD88. IRAK-2 is required for the activation of NF $\kappa$ B by MAL, but not IRAK, whereas MyD88 requires both IRAKs. MAL associates with IRAK-2 by its TIR domain. In addition, MAL 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.
5. Horng, T., et al. 2001. TIRAP: an adapter molecule in the Toll signaling pathway. *Nat. Immunol.* 2: 835-841.

## CHROMOSOMAL LOCATION

Genetic locus: Tirap (mouse) mapping to 9 A4.

## PRODUCT

TIRAP shRNA (m) 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see TIRAP siRNA (m): sc-44740 and TIRAP shRNA Plasmid (m): sc-44740-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

TIRAP shRNA (m) Lentiviral Particles is recommended for the inhibition of TIRAP expression in mouse cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

TIRAP (FL-235): sc-28822 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TIRAP gene expression knockdown using RT-PCR Primer: TIRAP (m)-PR: sc-44740-PR (20  $\mu$ l, 497 bp). Annealing temperature for the primers should be  $55-60^{\circ}$  C and the extension temperature should be  $68-72^{\circ}$  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.

## PROTOCOLS

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