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MyD88 shRNA (h2) Lentiviral Particles: sc-44313-V

BACKGROUND

Interleukin-1 (IL-1)-induced activation of the NF κ B pathway is mediated through the IL-1 receptor and the subsequent phosphorylation of IL-1 receptor-associated kinase (IRAK). The myeloid differentiation protein MyD88 was originally characterized as a protein upregulated in myeloleukemic cells following IL-6-induced growth arrest and terminal differentiation. MyD88 is now known to function as an adaptor protein for the association of IRAK with the IL-1 receptor. MyD88 is functionally homologous to the adaptor protein tube in the Toll signaling pathway of *Drosophila*, and both proteins are members of the Toll/IL-1R superfamily. MyD88 contains a characteristic N-terminal death domain that is essential for NF κ B activation and an adjacent Toll/IL-1R homology domain (TIR domain). Collectively, these domains enable the protein-protein interactions of MyD88 with IRAK and the IL-1 receptor complex.

REFERENCES

- Galindo, R.L., et al. 1995. Interaction of the pelle kinase with the membrane-associated protein tube is required for transduction of the dorsoventral signal in *Drosophila* embryos. *Development* 121: 2209-2218.
- Hardiman, G., et al. 1996. Molecular characterization and modular analysis of human MyD88. *Oncogene* 13: 2467-2475.
- Muzio, M., et al. 1997. IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science* 278: 1612-1615.
- Wesche, H., et al. 1997. MyD88: an adaptor that recruits IRAK to the IL-1 receptor complex. *Immunity* 7: 837-847.
- Adachi, O., et al. 1998. Targeted disruption of the MyD88 gene results in loss of IL-1 and IL-18-mediated function. *Immunity* 9: 143-150.

CHROMOSOMAL LOCATION

Genetic locus: MYD88 (human) mapping to 3p22.2.

PRODUCT

MyD88 shRNA (h2) 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 μ l frozen stock containing 1.0×10^6 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 MyD88 siRNA (h2): sc-44313 and MyD88 shRNA Plasmid (h2): sc-44313-SH as alternate gene silencing products.

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.

APPLICATIONS

MyD88 shRNA (h2) Lentiviral Particles is recommended for the inhibition of MyD88 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

MyD88 (HFL-296): sc-11356 is recommended as a control antibody for monitoring of 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 MyD88 gene expression knockdown using RT-PCR Primer: MyD88 (h2)-PR: sc-44313-PR (20 μ l, 425 bp). 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.

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.

PROTOCOLS

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