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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

MyD88 siRNA (h2): sc-44313

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 siRNA (h2) 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 MyD88 shRNA Plasmid (h2): sc-44313-SH and MyD88 shRNA (h2) Lentiviral Particles: sc-44313-V as alternate gene silencing products.

For independent verification of MyD88 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3 nmol of lyophilized siRNA. These include: sc-44313A, sc-44313B and sc-44313C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

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

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

MyD88 siRNA (h2) is recommended for the inhibition of MyD88 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

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 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.