

SIMPL siRNA (h): sc-95101

BACKGROUND

SIMPL, also known as IRAK1BP1 (interleukin-1 receptor-associated kinase 1-binding protein 1), is a 260 amino acid protein belonging to the IRAK1BP1 family. In addition to containing an intrinsically disordered region which associates with HSPA1 and HSPA8, SIMPL also interacts directly with IRAK1 and promotes RELA transcriptional activity. SIMPL is a major component of the IRAK1-dependent TNFRSF1A signaling pathway in which TNF- α induces NF κ B gene transcription, leading to an inhibition of proinflammatory cytokines. Inactivation of SIMPL inhibits tumor necrosis factor receptor type I-induced NF κ B activity. Localizing to the nucleus and cytoplasm, SIMPL requires the phosphorylation of Ser 56 and/or Ser 62 to be fully active.

REFERENCES

1. Vig, E., Green, M., Liu, Y., Yu, K.Y., Kwon, H.J., Tian, J., Goebel, M.G. and Harrington, M.A. 2001. SIMPL is a tumor necrosis factor-specific regulator of nuclear factor- κ B activity. *J. Biol. Chem.* 276: 7859-7866.
2. Querengässer, J., Geishauser, T., Querengässer, K., Bruckmaier, R. and Fehlings, K. 2002. Comparative evaluation of SIMPL silicone implants and NIT natural teat inserts to keep the teat canal patent after surgery. *J. Dairy Sci.* 85: 1732-1737.
3. Kwon, H.J., Breese, E.H., Vig-Varga, E., Luo, Y., Lee, Y., Goebel, M.G. and Harrington, M.A. 2004. Tumor necrosis factor α induction of NF κ B requires the novel coactivator SIMPL. *Mol. Cell. Biol.* 24: 9317-9326.
4. Haag Breese, E., Uversky, V.N., Georgiadis, M.M. and Harrington, M.A. 2006. The disordered amino-terminus of SIMPL interacts with members of the 70-kDa heat-shock protein family. *DNA Cell Biol.* 25: 704-714.
5. Luo, Y., Kwon, H.J., Montano, S., Georgiadis, M., Goebel, M.G. and Harrington, M.A. 2007. Phosphorylation of SIMPL modulates RelA-associated NF κ B-dependent transcription. *Am. J. Physiol., Cell Physiol.* 292: C1013-C1023.
6. Conner, J.R., Smirnova, I.I. and Poltorak, A. 2008. Forward genetic analysis of Toll-like receptor responses in wild-derived mice reveals a novel anti-inflammatory role for IRAK1BP1. *J. Exp. Med.* 205: 305-314.
7. Benson, E.A., Goebel, M.G., Yang, F.C., Kapur, R., McClintick, J., Sanghani, S., Clapp, D.W. and Harrington, M.A. 2010. Loss of SIMPL compromises TNF- α -dependent survival of hematopoietic progenitors. *Exp. Hematol.* 38: 71-81.
8. Conner, J.R., Smirnova, I.I., Moseman, A.P. and Poltorak, A. 2010. IRAK1BP1 inhibits inflammation by promoting nuclear translocation of NF κ B p50. *Proc. Natl. Acad. Sci. USA* 107: 11477-11482.

CHROMOSOMAL LOCATION

Genetic locus: IRAK1BP1 (human) mapping to 6q14.1.

PROTOCOLS

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

PRODUCT

SIMPL 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 SIMPL shRNA Plasmid (h): sc-95101-SH and SIMPL shRNA (h) Lentiviral Particles: sc-95101-V as alternate gene silencing products.

For independent verification of SIMPL (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-95101A, sc-95101B and sc-95101C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° 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

SIMPL siRNA (h) is recommended for the inhibition of SIMPL 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor SIMPL gene expression knockdown using RT-PCR Primer: SIMPL (h)-PR: sc-95101-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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