

# Tak1L siRNA (h): sc-91477

## BACKGROUND

Several serine/threonine protein kinases have been implicated as intermediates in signal transduction pathways. These include ERK/MAP kinases, ribosomal S6 kinase (Rsk) and Raf-1. Raf-1 is a protein with intrinsic kinase activity towards serine/threonine residues and is widely expressed in many tissue types and cell lines. Raf-1 activation is dependent on the small molecular weight GTPase Ras. Two proteins putatively involved in this process are Ksr-1 and Tak1. Ksr-1 (kinase suppressor of Ras) is a novel Raf-related protein kinase whose function is required for Ras signal transduction. Whether Ksr-1 lies directly downstream of Ras or acts in a parallel pathway is not yet known. Tak1 (TGF $\beta$ -activated kinase) has been shown to participate in the activation of the MAP kinase family in response to TGF $\beta$  stimulation. Tak1L (Tak1-like protein), also known as C21orf7, is a 242 amino acid protein that shares homology with the C-terminal tail of Tak1. Tak1L is expressed predominantly in peripheral blood leukocytes, with strong expression found in the adenocarcinoma cell lines GI-112 and PC-3 and the carcinoma cell line GI-101.

## REFERENCES

- Huleihel, M., et al. 1986. Characterization of murine A-Raf, a new oncogene related to the v-Raf oncogene. *Mol. Cell. Biol.* 6: 2655-2662.
- Ray, L.B. and Sturgill, T.W. 1988. Insulin-stimulated microtubule-associated protein kinase is phosphorylated on tyrosine and threonine *in vivo*. *Proc. Natl. Acad. Sci. USA* 85: 3753-3757.
- Morrison, D.K., et al. 1988. Signal transduction from membrane to cytoplasm: growth factors and membrane-bound oncogene products increase Raf-1 phosphorylation and associated protein kinase activity. *Proc. Natl. Acad. Sci. USA* 85: 8855-8859.
- Pelech, S.L., et al. 1990. Protein kinase cascades in meiotic and mitotic cell cycle control. *Biochem. Cell Biol.* 68: 1297-1330.
- Downward, J. 1995. Ksr: a novel player in the Ras pathway. *Cell* 83: 831-834.
- Sundaram, M. and Han, M. 1995. The *C. elegans* Ksr-1 gene encodes a novel Raf-related kinase involved in Ras-mediated signal transduction. *Cell* 83: 889-901.

## CHROMOSOMAL LOCATION

Genetic locus: C21orf7 (human) mapping to 21q21.3.

## PRODUCT

Tak1L 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Tak1L shRNA Plasmid (h): sc-91477-SH and Tak1L shRNA (h) Lentiviral Particles: sc-91477-V as alternate gene silencing products.

For independent verification of Tak1L (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-91477A, sc-91477B and sc-91477C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

Tak1L siRNA (h) is recommended for the inhibition of Tak1L 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  $\mu$ M in 66  $\mu$ 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

Tak1L (G-8): sc-515478 is recommended as a control antibody for monitoring of Tak1L 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 Tak1L gene expression knockdown using RT-PCR Primer: Tak1L (h)-PR: sc-91477-PR (20  $\mu$ 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.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.