SANTA CRUZ BIOTECHNOLOGY, INC.

# SIAH-2 siRNA (m): sc-37498

## BACKGROUND

SIAH-2 (seven in absentia homolog 2) is an E3 ligase that catalyzes ubiquitination and proteasome-mediated degradation of protein substrates. SIAH-2 encodes a 324 amino acid protein that shares 77% identity with human SIAH-1 and 68% identity with the *Drosophila* sina (seven in absentia) gene, on which development of the *Drosophila* R7 photoreceptor is dependent. SIAH-2 targets TRAF2, which regulates cell responses to stress and cytokines through the regulation of key stress-signaling cascades, for degradation under stress conditions such as hypoxia. It targets HIF-1α prolyl hydroxylase 3 (PHD3) for degradation upon exposure to hypoxic conditions, which coincides with an increase in SIAH-2 transcription. SIAH-2 can decrease TNFα-dependent induction of JNK activity and transcriptional activation of NFκB. SIAH-2 null mice subjected to hypoxia display an impaired respiratory response and reduced levels of hemoglobin.

## REFERENCES


1. Della, N.G., et al. 1995. Expression of SIAH-2, a vertebrate homologue of *Drosophila* sina, in germ cells of the mouse ovary and testis. Cell Tissue Res. 279: 411-419.
2. Habelhah, H., et al. 2002. Stress-induced decrease in TRAF2 stability is mediated by SIAH-2. EMBO J. 21: 5756-5765.
3. Frew, I.J., et al. 2002. Normal p53 function in primary cells deficient for Siah genes. Mol. Cell. Biol. 22: 8155-8164.
4. Frew, I.J., et al. 2003. Generation and analysis of SIAH-2 mutant mice. Mol. Cell. Biol. 23: 9150-9161.
5. Simon, M.C. 2004. Siah proteins, HIF prolyl hydroxylases, and the physiological response to hypoxia. Cell 117: 851-853.
6. Nakayama, K., et al. 2004. SIAH-2 regulates stability of prolyl-hydroxylases, controls HIF1α abundance, and modulates physiological responses to hypoxia. Cell 117: 941-952.
7. Avraham, E., et al. 2005. Glycogen synthase kinase 3β modulates synphilin-1 ubiquitylation and cellular inclusion formation by SIAH: implications for proteasomal function and Lewy body formation. J. Biol. Chem. 280: 42877-42886.


## CHROMOSOMAL LOCATION

Genetic locus: Siah2 (mouse) mapping to 3 D.

## PRODUCT

SIAH-2 siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SIAH-2 shRNA Plasmid (m): sc-37498-SH and SIAH-2 shRNA (m) Lentiviral Particles: sc-37498-V as alternate gene silencing products.

For independent verification of SIAH-2 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-37498A, sc-37498B and sc-37498C.

## 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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

SIAH-2 siRNA (m) is recommended for the inhibition of SIAH-2 expression in mouse 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 µM in 66 µl. 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

SIAH-2 (24E6H3): sc-81787 is recommended as a control antibody for monitoring of SIAH-2 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 SIAH-2 gene expression knockdown using RT-PCR Primer: SIAH-2 (m)-PR: sc-37498-PR (20 µl). 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 for detailed protocols and support products.