

# HACE1 siRNA (h): sc-95301

## BACKGROUND

Ubiquitination is an important mechanism through which three classes of enzymes act in concert to target short-lived or abnormal proteins for destruction. The three classes of enzymes involved in ubiquitination are the ubiquitin-activating enzymes (E1s), the ubiquitin-conjugating enzymes (E2s) and the ubiquitin-protein ligases (E3s). HACE1 (HECT domain and ankyrin repeat containing, E3 ubiquitin-protein ligase 1), also known as KIAA1320, is a 909 amino acid protein that localizes to both the cytoplasm and the endoplasmic reticulum and contains one HECT domain and six ANK repeats. Expressed in kidney, heart and brain, HACE1 functions as an E3 ubiquitin-protein ligase that interacts with the proteasome and is thought to play a role in protein degradation. HACE1 is downregulated in Wilms tumor, suggesting a possible role in tumor suppression.

## REFERENCES

1. Nagase, T., et al. 2000. Prediction of the coding sequences of unidentified human genes. XVI. The complete sequences of 150 new cDNA clones from brain which code for large proteins *in vitro*. DNA Res. 7: 65-73.
2. Fernandez, C.V., et al. 2001. Detection of a novel t(6;15)(q21;q21) in a pediatric Wilms tumor. Cancer Genet. Cytogenet. 129: 165-167.
3. Anglesio, M.S., et al. 2004. Differential expression of a novel Ankyrin containing E3 ubiquitin-protein ligase, HACE1, in sporadic Wilms' tumor versus normal kidney. Hum. Mol. Genet. 13: 2061-2074.
4. Zhang, L., et al. 2007. The E3 ligase HACE1 is a critical chromosome 6q21 tumor suppressor involved in multiple cancers. Nat. Med. 13: 1060-1069.

## CHROMOSOMAL LOCATION

Genetic locus: HACE1 (human) mapping to 6q16.3.

## PRODUCT

HACE1 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 HACE1 shRNA Plasmid (h): sc-95301-SH and HACE1 shRNA (h) Lentiviral Particles: sc-95301-V as alternate gene silencing products.

For independent verification of HACE1 (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-95301A, sc-95301B and sc-95301C.

## 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

HACE1 siRNA (h) is recommended for the inhibition of HACE1 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

HACE1 (C-9): sc-515746 is recommended as a control antibody for monitoring of HACE1 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<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> 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<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HACE1 gene expression knockdown using RT-PCR Primer: HACE1 (h)-PR: sc-95301-PR (20  $\mu$ l, 411 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. El-Hachem, N., et al. 2018. Uncovering and deciphering the pro-invasive role of HACE1 in melanoma cells. Cell Death Differ. 25: 2010-2022.

## 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.