

# ALKBH3 siRNA (h): sc-96711

## BACKGROUND

ALKBH3 (alkB, alkylation repair homolog 3), also known as ABH3, PCA-1 (prostate cancer antigen-1) or DEPC-1, is a 286 amino acid member of the ALKB family of proteins and functions as a dioxygenase with a preference for RNA and single stranded DNA substrates. ALKBH3 is one of many homologs of the *Escherichia coli* protein, ALKB. ALKBH3 is expressed in a wide variety of tissues and localizes to the cytoplasm and the nucleus. It associates with iron and 2-oxoglutarate, coupling the oxidation of substrates to the conversion of 2-oxoglutarate into succinate and CO<sub>2</sub>. Via oxidative demethylation, ALKBH3 repairs 1-methyladenine and 3-methylcytosine lesions in alkylated DNA and RNA. Its activity is stimulated by ascorbate. Two isoforms exist for ALKBH3 due to alternative splicing of the gene.

## REFERENCES

1. Duncan, T., et al. 2002. Reversal of DNA alkylation damage by two human dioxygenases. *Proc. Natl. Acad. Sci. USA* 99: 16660-16665.
2. Online Mendelian Inheritance in Man, OMIM<sup>™</sup>. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 610603. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Ougland, R., et al. 2004. ALKB restores the biological function of mRNA and tRNA inactivated by chemical methylation. *Mol. Cell* 16: 107-116.
4. Mishina, Y., et al. 2004. Interaction of human and bacterial ALKB proteins with DNA as probed through chemical cross-linking studies. *Nucleic Acids Res.* 32: 1548-1554.
5. Falnes, P.O., et al. 2004. Substrate specificities of bacterial and human ALKB proteins. *Nucleic Acids Res.* 32: 3456-3461.
6. Konishi, N., et al. 2005. High expression of a new marker PCA-1 in human prostate carcinoma. *Clin. Cancer Res.* 11: 5090-5097.
7. Ringvoll, J., et al. 2006. Repair deficient mice reveal mABH2 as the primary oxidative demethylase for repairing 1meA and 3meC lesions in DNA. *EMBO J.* 25: 2189-2198.
8. Sundheim, O., et al. 2006. Human ABH3 structure and key residues for oxidative demethylation to reverse DNA/RNA damage. *EMBO J.* 25: 3389-3397.

## CHROMOSOMAL LOCATION

Genetic locus: ALKBH3 (human) mapping to 11p11.2.

## PRODUCT

ALKBH3 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 ALKBH3 shRNA Plasmid (h): sc-96711-SH and ALKBH3 shRNA (h) Lentiviral Particles: sc-96711-V as alternate gene silencing products.

For independent verification of ALKBH3 (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-96711A, sc-96711B and sc-96711C.

## 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

ALKBH3 siRNA (h) is recommended for the inhibition of ALKBH3 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

ALKBH3 (B-7): sc-376520 is recommended as a control antibody for monitoring of ALKBH3 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 ALKBH3 gene expression knockdown using RT-PCR Primer: ALKBH3 (h)-PR: sc-96711-PR (20  $\mu$ l, 518 bp). 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.