

ALKBH2 siRNA (h): sc-96063

BACKGROUND

ALKBH2 (alkB, alkylation repair homolog 2), also known as ABH2, is a 261 amino acid protein that localizes to the nucleus and belongs to the ALKB family. Expressed in heart, colon, liver, testis, ovary, prostate and small intestine, ALKBH2 uses iron as a cofactor and functions as a dioxygenase that catalyzes the repair of alkylated DNA and RNA containing 1-methyladenine and 3-methylcytosine. ALKBH2 is functionally activated by ascorbate and requires oxygen and α -ketoglutarate for enzymatic activity. The gene encoding ALKBH2 maps to human chromosome 12, which encodes over 1,100 genes and comprises approximately 4.5% of the human genome. Chromosome 12 is associated with a variety of diseases and afflictions, including hypochondrogenesis, achondrogenesis, Kniest dysplasia, Noonan syndrome and Trisomy 12p, which causes facial developmental defects and seizure disorders.

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. Aas, P.A., et al. 2003. Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA. *Nature* 421: 859-863.
3. Lee, D.H., et al. 2005. Repair of methylation damage in DNA and RNA by mammalian ALKB homologues. *J. Biol. Chem.* 280: 39448-39459.
4. 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.
5. Ringvoll, J., et al. 2008. ALKB homologue 2-mediated repair of ethenoadenine lesions in mammalian DNA. *Cancer Res.* 68: 4142-4149.

CHROMOSOMAL LOCATION

Genetic locus: ALKBH2 (human) mapping to 12q24.11.

PRODUCT

ALKBH2 siRNA (h) is a pool of 2 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 ALKBH2 shRNA Plasmid (h): sc-96063-SH and ALKBH2 shRNA (h) Lentiviral Particles: sc-96063-V as alternate gene silencing products.

For independent verification of ALKBH2 (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-96063A and sc-96063B.

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

ALKBH2 siRNA (h) is recommended for the inhibition of ALKBH2 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.

GENE EXPRESSION MONITORING

ALKBH2 (C-9): sc-515789 is recommended as a control antibody for monitoring of ALKBH2 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 ALKBH2 gene expression knockdown using RT-PCR Primer: ALKBH2 (h)-PR: sc-96063-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.

PROTOCOLS

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