SANTA CRUZ BIOTECHNOLOGY, INC.

ALKB siRNA (h): sc-60153

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

ALKB protects DNA and RNA against damage from methylating compounds from the environment by directly reversing 1-methyladenine (1-meA) and 3-methylcytosine (3-meC) cytotoxic alkylation lesions in DNA and RNA. The enzymes act by oxidative demethylation, utilizing ferrous iron and α-ketoglutarate as cofactors, 2-oxoglutarate as a co-substrate, and molecular oxygen as the oxidizing agent. Deficiencies in DNA and RNA repair in mammals are associated with cancer, neurological disease and developmental defects. ALKB plays a role in resistance to anti-cancer drugs which attempt to damage tumor DNA. *Escherichia coli* ALKB protein belongs to the superfamily of 2-oxoglutarate- and iron(II)-dependent oxygenases.

REFERENCES

1. Ougland, R., et al. 2004. ALKB restores the biological function of mRNA and tRNA inactivated by chemical methylation. Mol. Cell 16: 107-116.
2. Drablos, F., et al. 2004. Alkylation damage in DNA and RNA—repair mechanisms and medical significance. DNA Repair 3: 1389-1407.
3. Falnes, P.O. 2004. Repair of 3-methylthymine and 1-methylguanine lesions by bacterial and human ALKB proteins. Nucleic Acids Res. 32: 6260-6267.
4. Koivisto, P., et al. 2004. Demethylation of 3-methylthymine in DNA by bacterial and human DNA dioxygenases. J. Biol. Chem. 279: 40470-40474.
5. Henshaw, T.F., et al. 2004. Aberrant activity of the DNA repair enzyme ALKB. J. Inorg. Biochem. 98: 856-861.
6. Sedgwick, B., et al. 2006. Direct removal of alkylation damage from DNA by ALKB and related DNA dioxygenases. Methods Enzymol. 408: 108-120.

CHROMOSOMAL LOCATION

Genetic locus: ALKBH1 (human) mapping to 14q24.3.

PRODUCT

ALKB 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ALKB shRNA Plasmid (h): sc-60153-SH and ALKB shRNA (h) Lentiviral Particles: sc-60153-V as alternate gene silencing products.

For independent verification of ALKB (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-60153A, sc-60153B and sc-60153C.

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

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

ALKB (F-4): sc-374301 is recommended as a control antibody for monitoring of ALKB 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 ALKB gene expression knockdown using RT-PCR Primer: ALKB (h)-PR: sc-60153-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Wu, L., et al. 2019. Association of N^6-methyladenine DNA with plaque progression in atherosclerosis via myocardial infarction-associated transcripts. Cell Death Dis. 10: 909.
2. Liu, Y., et al. 2022. DNA demethylase ALKBH1 promotes adipogenic differentiation via regulation of HIF-1 signaling. J. Biol. Chem. 298: 101499.

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.