SANTA CRUZ BIOTECHNOLOGY, INC.

OPLAH siRNA (h): sc-77750

BACKGROUND

The γ-glutamyl cycle involves a series of reactions that are necessary for the synthesis and metabolism of glutathione (GST), which is crucial for regulating amino acid transport. OPLAH [5-oxoprolinase (ATP-hydrolysing)], also known as OPLA, 5-oxo-L-prolinase, 5-Opase or DKFZp434H244OPLA, is an enzyme that plays an important role in the γ-glutamyl cycle by catalyzing the cleavage of 5-oxo-L-proline to form L-glutamate in a reaction coupled to the hydrolysis of ATP to ADP and inorganic phosphate. OPLAH is a 1,288 amino acid protein that exists as a homodimer and belongs to the oxoprolinase family. Expressed at highest levels in kidney, OPLAH has also been found at lower levels in lung, breast, colon and ovary. The gene encoding OPLAH maps to human chromosome 8, which consists of nearly 146 million base pairs, encodes over 800 genes and is associated with a variety of diseases and malignancies including Schizophrenia, bipolar disorder, Trisomy 8, Pfeiffer syndrome and congenital hypothyroidism.

REFERENCES

1. Srivenugopal, K.S., et al. 1997. Activity and distribution of the cysteine prodrug activating enzyme, 5-oxo-L-prolinase, in human normal and tumor tissues. Cancer Lett. 117: 105-111.
2. Chen, X., et al. 1998. Characterization of 5-oxo-L-prolinase in normal and tumor tissues of humans and rats: a potential new target for biochemical modulation of glutathione. Clin. Cancer Res. 4: 131-138.
3. Jäger, M., et al. 1999. Localization of 5-oxo-L-prolinase mRNA in the murine choroid plexus by *in situ* hybridization. Neurosci. Lett. 274: 171-174.
4. Kashino, G., et al. 2001. Preferential expression of an intact WRN gene in Werner syndrome cell lines in which a normal chromosome 8 has been introduced. Biochem. Biophys. Res. Commun. 289: 111-115.
5. Selicorni, A., et al. 2002. Cytogenetic mapping of a novel locus for type II Waardenburg syndrome. Hum. Genet. 110: 64-67.
6. Watanabe, T., et al. 2004. Bovine 5-oxo-L-prolinase: simple assay method, purification, cDNA cloning, and detection of mRNA in the coronary artery. Biol. Pharm. Bull. 27: 288-294.

CHROMOSOMAL LOCATION

Genetic locus: OPLAH (human) mapping to 8q24.3.

PRODUCT

OPLAH 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 OPLAH shRNA Plasmid (h): sc-77750-SH and OPLAH shRNA (h) Lentiviral Particles: sc-77750-V as alternate gene silencing products.

For independent verification of OPLAH (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-77750A, sc-77750B and sc-77750C.

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

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

OPLAH (C-6): sc-393570 is recommended as a control antibody for monitoring of OPLAH 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 OPLAH gene expression knockdown using RT-PCR Primer: OPLAH (h)-PR: sc-77750-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.