SANTA CRUZ BIOTECHNOLOGY, INC.

kynureninase siRNA (h): sc-95023

BACKGROUND

Kynureninase, also known as L-kynurenine hydrolase, is a 465 amino acid cytoplasmic enzyme. Kynureninase is involved in two pathways; the degredation of L-kynurenine and the biosynthesis of the cofactor NAD^+. The main function of kynureninase is to catalyze the cleavage of L-kynurenine into anthranilic acid and of L-3-hydroxykynurenine into 3-hydroxyanthranilic acid, exhibiting a preference for the L-3-hydroxy form. Kynureninase forms a homodimer, uses pyridoxal phosphate as a cofactor and is inhibited by o-methoxybenzoylalanine (OMBA). Kynureninase is widely expressed, with highest levels found in lung, placenta and liver. Deficiency in kynureninase leads to hyperkynureninuria, a disorder characterized by the inability to break down tryptophan to nicotinic acid (vitamin B6). Increased levels of kynureninase activity are observed in systemic and cerebral inflammatory conditions.

REFERENCES

1. Komrower, G.M., et al. 1964. Hydroxykynureninuria: a case of abnormal tryptophane metabolism probably due to a deficiency of kynureninase. Arch. Dis. Child. 39: 250-256.
2. Heyes, M.P., et al. 1993. A mechanism of quinolinic acid formation by brain in inflammatory neurological disease. Attenuation of synthesis from L-tryptophan by 6-chlorotryptophan and 4-chloro-3-hydroxyanthranilate. Brain 116: 1425-1450.
3. Alberati-Giani, D., et al. 1996. Isolation and expression of a cDNA clone encoding human kynureninase. Eur. J. Biochem. 239: 460-468.
4. Cheminal, R., et al. 1996. Congenital non-progressive encephalopathy and deafness with intermittent episodes of coma and hyperkynureninuria. J. Inherit. Metab. Dis. 19: 25-30.
5. Toma, S., et al. 1997. Cloning and recombinant expression of rat and human kynureninase. FEBS Lett. 408: 5-10.

CHROMOSOMAL LOCATION

Genetic locus: KYNU (human) mapping to 2q22.2.

PRODUCT

kynureninase 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 kynureninase shRNA Plasmid (h): sc-95023-SH and kynureninase shRNA (h) Lentiviral Particles: sc-95023-V as alternate gene silencing products.

For independent verification of kynureninase (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-95023A, sc-95023B and sc-95023C.

PROTOCOLS

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

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

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

kynureninase (E-5): sc-390360 is recommended as a control antibody for monitoring of kynureninase 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 kynureninase gene expression knockdown using RT-PCR Primer: kynureninase (h)-PR: sc-95023-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.