SANTA CRUZ BIOTECHNOLOGY, INC.

HPRT siRNA (h): sc-40679

BACKGROUND

HPRT (hypoxanthine phosphoribosyltransferase 1), also known as HGPRT or HPRT1, is a 218 amino acid cytoplasmic protein that belongs to the purine/pyrimidine phosphoribosyltransferase family. Involved in purine metabolism, HPRT functions as a purine salvage enzyme that catalyzes the conversion of hypoxathine and guanine to their respective mononucleotides (inosine monophosphate and guanosine monophosphate, respectively). HPRT exists as a homotetramer that can bind two magnesium ions as cofactors. Defects in the gene encoding HPRT are the cause of gout and Lesch-Nyhan syndrome (LNS), both of which are characterized by a partial or complete lack of NPRT enzymatic activity. While a partial loss of HPRT enzymatic activity results in a buildup of uric acid (gout), a total loss of enzymatic activity results in hyperuricaemia, mental retardation, choreoathetosis and compulsive self-mutilation, all of which are symptoms associated with LNS. The severity of these diseases suggests an essential role for HPRT in purine metabolism.

REFERENCES

1. Stout, J.T., et al. 1985. HPRT: gene structure, expression, and mutation. Annu. Rev. Genet. 19: 127-148.
2. Fujimori, S., et al. 1997. An asymptomatic germline missense base substitution in the hypoxanthine phosphoribosyltransferase (HPRT) gene that reduces the amount of enzyme in humans. Hum. Genet. 99: 8-10.
3. Mizunuma, M., et al. 2001. A recurrent large Alu-mediated deletion in the hypoxanthine phosphoribosyltransferase (HPRT1) gene associated with Lesch-Nyhan syndrome. Hum. Mutat. 18: 435-443.
4. Koina, E., et al. 2005. An inactive X specific replication origin associated with a matrix attachment region in the human X linked HPRT gene. J. Cell. Biochem. 95: 391-402.
5. Dawson, P.A., et al. 2005. Normal HPRT coding region in a male with gout due to HPRT deficiency. Mol. Genet. Metab. 85: 78-80.
6. Cossu, A., et al. 2006. HPRTSardinia: a new point mutation causing HPRT deficiency without Lesch-Nyhan disease. Biochim. Biophys. Acta 1762: 29-33.
7. Keebaugh, A.C., et al. 2007. Gene duplication and inactivation in the HPRT gene family. Genomics 89: 134-142.

CHROMOSOMAL LOCATION

Genetic locus: HPRT1 (human) mapping to Xq26.2.

PRODUCT

HPRT 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 HPRT shRNA Plasmid (h): sc-40679-SH and HPRT shRNA (h) Lentiviral Particles: sc-40679-V as alternate gene silencing products.

For independent verification of HPRT (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-40679A, sc-40679B and sc-40679C.

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

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

HPRT (F-1): sc-376938 is recommended as a control antibody for monitoring of HPRT 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 HPRT gene expression knockdown using RT-PCR Primer: HPRT (h)-PR: sc-40679-PR (20 µl, 583 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 for detailed protocols and support products.