SANTA CRUZ BIOTECHNOLOGY, INC.

GPT siRNA (h): sc-60753

BACKGROUND

The glutamate pyruvate transaminases GPT (or GPT1) and GPT2, also designated alanine aminotransferases (ALT1 and ALT2), respectively, catalyze the reversible transamination between alanine and 2-oxoglutarate to form pyruvate and glutamate. Subsequently, they play a key role in the intermediary metabolism of glucose and amino acids. GPT and GPT2 share significant sequence homology, but differ in their expression patterns. GPT exhibits high expression in kidney, liver and heart, whereas GPT2 expression is high in muscle, fat and kidney. GPT is widely used as an index of liver integrity or hepatocellular damage in clinical settings.

REFERENCES

1. Sohocki, M.M., et al. 1997. Human glutamate pyruvate transaminase (GPT): localization to 8q24.3, cDNA and genomic sequences and polymorphic sites. Genomics 40: 247-252.
2. Yang, R.Z., et al. 2002. cDNA cloning, genomic structure, chromosomal mapping and functional expression of a novel human alanine aminotransferase. Genomics 79: 445-450.
3. Matthews, C.C., et al. 2003. Glutamate-pyruvate transaminase protects against glutamate toxicity in hippocampal slices. Brain Res. 978: 59-64.
4. Jadhao, S.B., et al. 2004. Murine alanine aminotransferase: cDNA cloning, functional expression and differential gene regulation in mouse fatty liver. Hepatology 39: 1297-1302.
5. Lagoa, C.E., et al. 2005. The role of hepatic type 1 plasminogen activator inhibitor (PAI-1) during murine hemorrhagic shock. Hepatology 42: 390-399.
6. Nagel, S., et al. 2005. An improved model of isolated hemoperfused porcine livers using pneumatically driven pulsating blood pumps. Toxicol. Pathol. 33: 434-440.
7. Schindhelm, R.K., et al. 2005. Liver alanine aminotransferase, Insulin resistance and endothelial dysfunction in normotriglyceridaemic subjects with type 2 diabetes mellitus. Eur. J. Clin. Invest. 35: 369-374.

CHROMOSOMAL LOCATION

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

PRODUCT

GPT 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 GPT shRNA Plasmid (h): sc-60753-SH and GPT shRNA (h) Lentiviral Particles: sc-60753-V as alternate gene silencing products.

For independent verification of GPT (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-60753A, sc-60753B and sc-60753C.

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

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

GPT (E-3): sc-374501 is recommended as a control antibody for monitoring of GPT 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 GPT gene expression knockdown using RT-PCR Primer: GPT (h)-PR: sc-60753-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.