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GPT2 shRNA (m) Lentiviral Particles: sc-45648-V

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. Matthews, C.C., 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. Nagel, S., et al. 2005. An improved model of isolated hemoperfused porcine livers using pneumatically driven pulsating blood pumps. *Toxicol. Pathol.* 33: 434-440.

CHROMOSOMAL LOCATION

Genetic locus: Gpt2 (mouse) mapping to 8 C3.

PRODUCT

GPT2 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see GPT2 siRNA (m): sc-45648 and GPT2 shRNA Plasmid (m): sc-45648-SH as alternate gene silencing products.

APPLICATIONS

GPT2 shRNA (m) Lentiviral Particles is recommended for the inhibition of GPT2 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

GPT2 (G-7): sc-398383 is recommended as a control antibody for monitoring of GPT2 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GPT2 gene expression knockdown using RT-PCR Primer: GPT2 (m)-PR: sc-45648-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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