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Transketolase shRNA (h) Lentiviral Particles: sc-45591-V

BACKGROUND

Transketolase (TK or TKT), a member of the Transketolase family, is a multi-functional protein that plays a role in diabetes, cancer, Alzheimer's disease and Wernicke-Korsakoff's syndrome, a latent genetic neurological disorder. Transketolase is also important for the prevention of hyperglycemia-induced vascular damage. Transketolase is a crucial protein in the pentose phosphate pathway (PPP), where it catalyzes several reactions. In combination with Transaldolase, Transketolase functions as a link between glycolysis and the non-oxidative part of the PPP, allowing the cell to adapt to varying metabolic conditions in response to environmental changes. Transketolase activity is detected in small intestine epithelia, liver parenchyma, tongue, cornea and trachea. It is also expressed in the proximal tubules of kidney and in ganglion cells in medulla of the adrenal gland.

REFERENCES

1. Salamon, C., et al. 1998. The mouse transketolase (TKT) gene: cloning, characterization, and functional promoter analysis. *Genomics* 48: 209-220.
2. Kochetov, G.A., et al. 2001. Functional flexibility of the transketolase molecule. *Biochemistry* 66: 1077-1085.
3. Hammes, H.P., et al. 2003. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nat. Med.* 9: 294-299.
4. Babaei-Jadidi, R., et al. 2004. High-dose thiamine therapy counters dyslipidaemia in streptozotocin-induced diabetic rats. *Diabetologia* 47: 2235-2246.
5. Esakova, O.A., et al. 2004. Donor substrate regulation of Transketolase. *Eur. J. Biochem.* 271: 4189-4194.

CHROMOSOMAL LOCATION

Genetic locus: TKT (human) mapping to 3p21.1

PRODUCT

Transketolase shRNA (h) 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 Transketolase siRNA (h): sc-45591 and Transketolase shRNA Plasmid (h): sc-45591-SH as alternate gene silencing products.

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.

PROTOCOLS

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

APPLICATIONS

Transketolase shRNA (h) Lentiviral Particles is recommended for the inhibition of Transketolase expression in human 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

Transketolase (H-7): sc-390179 is recommended as a control antibody for monitoring of Transketolase 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 Transketolase gene expression knockdown using RT-PCR Primer: Transketolase (h)-PR: sc-45591-PR (20 μ l, 537 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.

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.