SANTA CRUZ BIOTECHNOLOGY, INC.

TIM siRNA (h): sc-37172

BACKGROUND

Glycolysis is an evolutionarily conserved series of 10 chemical reactions that utilizes 11 enzymes to concomitantly generate pyruvate and ATP from glucose. Triosephosphate isomerase, known as TIM or TPI, is ubiquitously expressed and catalyzes the interconversion of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate in the glycolitic pathway. The human TIM gene spans 3.5 kilobase pairs, contains seven exons and encodes a 249 amino acid protein. The TIM promoter element contains a TATA box (positions -27 to -21) and multiple GC boxes (positions -126 to -48) that variably conform to the consensus Sp1-binding site. The GC boxes function in *cis* to the TATA box to control both the frequency and position of transcription initiation. Deficiencies in TIM result in a rare autosomal recessive condition where a metabolic block in glycolysis and accumulating DHAP in erythrocytes can lead to non-spherocytic hemolytic anemia, recurrent infections, cardiomyopathy, and neuromuscular dysfunctions.

REFERENCES

1. Boyer, T.G., et al. 1989. Transcriptional regulatory sequences of the housekeeping gene for human triosephosphate isomerase. J. Biol. Chem. 264: 5177-5187.
2. Ansari-Lari, M.A., et al. 1996. A gene-rich cluster between the CD4 and triosephosphate isomerase genes at human chromosome 12p13. Genome Res. 6: 314-326.
3. Ansari-Lari, M.A., et al. 1997. Large-scale sequencing in human chromosome 12p13: experimental and computational gene structure determination. Genome Res. 7: 268-280.
4. Ationu, A., et al. 1998. The feasibility of replacement therapy for inherited disorder of glycolysis: triosephosphate isomerase deficiency. Int. J. Mol. Med. 2: 701-704.
5. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 190450. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
6. LocusLink Report (LocusID: 7167). http://www.ncbi.nlm. nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: TPI1 (human) mapping to 12p13.31.

PRODUCT

TIM 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 TIM shRNA Plasmid (h): sc-37172-SH and TIM shRNA (h) Lentiviral Particles: sc-37172-V as alternate gene silencing products.

For independent verification of TIM (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-37172A, sc-37172B and sc-37172C.

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

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

TIM (H-11): sc-166785 is recommended as a control antibody for monitoring of TIM 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 TIM gene expression knockdown using RT-PCR Primer: TIM (h)-PR: sc-37172-PR (20 µl, 524 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.