SANTA CRUZ BIOTECHNOLOGY, INC.

PDC-E2 siRNA (h): sc-40813

BACKGROUND

Primary biliary cirrhosis (PBC) is a chronic, destructive autoimmune liver disease characterized by the presence of antimitochondrial autoantibodies in patient's serum and T cell-mediated destruction of the biliary epithelial cells lining the small intrahepatic bile ducts. Patient sera are characterized by a high frequency (greater than 95%) of autoantibodies directed to a mitochondrial antigen, identified as the E2 component of the pyruvate dehydrogenase multienzyme complex (PDC-E2). PDC-E2 contains both an amino-terminal lipoyl-bearing domain and a carboxy-terminal catalytic domain. The human sequence preserves the Glu-Thr-Asp-Lys-Ala motif of the lipoyl-bearing site. Two conformationally alternative forms of the PDC-E2 protein have been revealed by immunoblotting. The immunodominant autoepitopes of the autoantigens correspond to the inner lipoyl domain. A significant number of asymptomatic patients found to have antibodies to PDC-E2 are at high risk of developing primary biliary cirrhosis.

REFERENCES

1. Coppel, R.L., et al. 1988. Primary structure of the human M2 mitochondrial autoantigen of primary biliary cirrhosis: dihydrolipoamide acetyltransferase. Proc. Natl. Acad. Sci. USA 85: 7317-7321.
2. Thekkumkara, T.J., et al. 1988. Nucelotide sequence of a cDNA for the dihydrolipoamide acetyltransferase component of human pyruvate dehydrogenase complex. FEBS Lett. 240: 45-48.
3. Klein, R., et al. 1993. Sera from patients with tuberculosis recognize the M2a-epitope (E2 subunit of pyruvate dehydrogenase) specific for primary biliary cirrhosis. Clin. Exp. Immunol. 92: 308-316.
4. Chen, Q.Y., et al. 1993. Antibody to two forms of dihydrolipoamide acetyltransferase (PDC-E2) in primary biliary cirrhosis. Liver 13: 130-135.
5. Howard, M.J., et al. 1998. Three-dimensional structure of the major autoantigen in primary biliary cirrhosis. Gastroenterology 115: 139-146.
6. Palmer, J.M., et al. 1999. T cell responses to the putative dominant autoepitope in primary biliary cirrhosis (PBC). Clin. Exp. Immunol. 116: 133-139.
7. Quaranta, S., et al. 1999. The immunopathogenesis of primary biliary cirrhosis. Hepatogastroenterology 46: 3041-3047.

CHROMOSOMAL LOCATION

Genetic locus: DLAT (human) mapping to 11q23.1.

PRODUCT

PDC-E2 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 PDC-E2 shRNA Plasmid (h): sc-40813-SH and PDC-E2 shRNA (h) Lentiviral Particles: sc-40813-V as alternate gene silencing products.

For independent verification of PDC-E2 (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-40813A, sc-40813B and sc-40813C.

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

PDC-E2 siRNA (h) is recommended for the inhibition of PDC-E2 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

PDC-E2 (B-2): sc-271534 is recommended as a control antibody for monitoring of PDC-E2 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 PDC-E2 gene expression knockdown using RT-PCR Primer: PDC-E2 (h)-PR: sc-40813-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.

PROTOCOLS

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