SANTA CRUZ BIOTECHNOLOGY, INC.

E3BP siRNA (m): sc-77213

BACKGROUND

The pyruvate dehydrogenase (PDH) complex is a nuclear-encoded mitochondrial matrix enzyme complex that functions as the primary link between glycolysis and the tricarboxylic acid (TCA) cycle by catalyzing the irreversible conversion of pyruvate into acetyl-CoA. E3BP (E3-binding protein), also known as PDHX (pyruvate dehydrogenase protein X component) and Lipoyl-containing pyruvate dehydrogenase complex component X, is a 501 amino acid mitochondrial protein that is required for anchoring E3 to the E2 core of the PDH complex, an event that is essential for a functional PDH complex. Defects in the gene encoding E3BP result in pyruvate dehydrogenase E3-binding protein deficiency, which is similar to PDH deficiency and Leigh syndrome in clinical presentation. Symptoms of E3BP deficiency can include lactic acidosis, delayed development, seizures, diplegia, cerebellar ataxia, optic atrophy, facial dysmorphism and episodic weakness.

REFERENCES

1. Robinson, B.H., et al. 1990. Defects in the E2 lipoyl transacetylase and the X-lipoyl containing component of the pyruvate dehydrogenase complex in patients with lactic acidemia. J. Clin. Invest. 85: 1821-1824.
2. Morava, E., et al. 2005. Mitochondrial dysfunction in a patient with Joubert syndrome. Neuropediatrics 36: 214-217.
3. Schiff, M., et al. 2006. Leigh's disease due to a new mutation in the PDHX gene. Ann. Neurol. 59: 709-714.
4. Brown, R.M., et al. 2006. Pyruvate dehydrogenase E3 binding protein (protein X) deficiency. Dev. Med. Child Neurol. 48: 756-760.
5. Smolle, M., et al. 2006. A new level of architectural complexity in the human pyruvate dehydrogenase complex. J. Biol. Chem. 281: 19772-19780.
6. McHugh, A., et al. 2006. PDC-E3BP is not a dominant T-cell autoantigen in primary biliary cirrhosis. Liver Int. 26: 406-413.
7. Miné, M., et al. 2006. A novel gross deletion caused by non-homologous recombination of the PDHX gene in a patient with pyruvate dehydrogenase deficiency. Mol. Genet. Metab. 89: 106-110.
8. Online Mendelian Inheritance in Man, OMIM™. 2008. Johns Hopkins University, Baltimore, MD. MIM Number: 608769. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: Pdhx (mouse) mapping to 2 E2.

PRODUCT

E3BP siRNA (m) 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 E3BP shRNA Plasmid (m): sc-77213-SH and E3BP shRNA (m) Lentiviral Particles: sc-77213-V as alternate gene silencing products.

For independent verification of E3BP (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-77213A, sc-77213B and sc-77213C.

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

E3BP siRNA (m) is recommended for the inhibition of E3BP expression in mouse 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

E3BP (H-6): sc-377255 is recommended as a control antibody for monitoring of E3BP 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 E3BP gene expression knockdown using RT-PCR Primer: E3BP (m)-PR: sc-77213-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.