SANTA CRUZ BIOTECHNOLOGY, INC.

FKRP siRNA (h): sc-60645

BACKGROUND

Fukutin-related protein (FKRP) is ubiquitously expressed, with highest expression in heart, skeletal muscle and placenta, and weakest expression in lung, liver, brain, kidney and pancreas. FKRP localizes to the medial Golgi apparatus through its N-terminal and transmembrane domains. It is a predicted glycosyltransferase protein that plays a role in α-dystroglycan glycosylation. Mutations in FKRP cause various diseases including congenital muscular dystrophy 1C (MDC1C), limb-girdle muscular dystrophy type 2I (LGMD2I) and congenital muscular dystrophies (CMDs) with brain malformations and mental retardation. FKRP mutations may also cause muscle-eye-brain disease (MEB) and Walker-Warburg syndrome (WWS), disorders characterized by disruption of brain and eye structure in addition to muscular dystrophy. Mislocalization of FKRP from the Golgi apparatus is a potential result of mutations in FKRP.

REFERENCES

1. Brockington, M., et al. 2001. Mutations in the Fukutin-related protein gene muscular dystrophy with secondary Laminin α2 deficiency and abnormal glycosylation of α-dystroglycan. Am. J. Hum. Genet. 69: 1198-1209.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606596. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
3. Esapa, C.T., et al. 2005. Fukutin-related protein mutations that cause congenital muscular dystrophy result in ER-retention of the mutant protein in cultured cells. Hum. Mol. Genet. 14: 295-305.
4. Müller, T., et al. 2005. Dilated cardiomyopathy may be an early sign of the C826A Fukutin-related protein mutation. Neuromuscul. Disord. 15: 372-376.
5. Dolatshad, N.F., et al. 2005. Mutated Fukutin-related protein (FKRP) localises as wildtype in differentiated muscle cells. Exp. Cell Res. 309: 370-378.
6. Boito, C.A., et al. 2005. Clinical and molecular characterization of patients with limb-girdle muscular dystrophy type 2I. Arch. Neurol. 62: 1894-1899.
7. Vajsar, J. and Schachter, H. 2006. Walker-Warburg syndrome. Orphanet J. Rare Dis. 1: 29.

CHROMOSOMAL LOCATION

Genetic locus: FKRP (human) mapping to 19q13.32.

PRODUCT

FKRP 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 FKRP shRNA Plasmid (h): sc-60645-SH and FKRP shRNA (h) Lentiviral Particles: sc-60645-V as alternate gene silencing products.

For independent verification of FKRP (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-60645A, sc-60645B and sc-60645C.

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

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

FKRP (E-4): sc-374642 is recommended as a control antibody for monitoring of FKRP 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 FKRP gene expression knockdown using RT-PCR Primer: FKRP (h)-PR: sc-60645-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.