SANTA CRUZ BIOTECHNOLOGY, INC.

Riboflavin kinase siRNA (m): sc-62941

BACKGROUND

Riboflavin kinase, also known as RFK or RIFK, is a cytoplasmic protein that catalyzes the first step in flavocoenzyme biosynthesis, namely the ATP-dependent phosphorylation of riboflavin to form flavin-mononucleotide (FMN). Expressed in the brain, placenta and bladder, Riboflavin kinase is a 162 amino acid protein for which zinc and magnesium are cofactors. Riboflavin kinase has three distinct conformational states that are referred to as the binary MgADP complex, the ternary product complex and the apo form, all of which contribute to the unique substrate binding and catalytic activity of the enzyme. Human Riboflavin kinase shares 44% homology with its yeast counterpart, suggesting that the three flexible regions surrounding the active site (termed Flap I, Flap II and Helix B) are similar in both species.

REFERENCES

1. Barile, M., et al. 2000. The riboflavin/FAD cycle in rat liver mitochondria. Eur. J. Biochem. 267: 4888-4900.
2. Karthikeyan, S., et al. 2003. Crystal structure of human riboflavin kinase reveals a β barrel fold and a novel active site arch. Structure 11: 265-273.
3. Karthikeyan, S., et al. 2003. Ligand binding-induced conformational changes in riboflavin kinase: structural basis for the ordered mechanism. Biochemistry 42: 12532-12538.
4. Solovieva, I.M., et al. 2005. The riboflavin kinase encoding gene ribR of *Bacillus subtilis* is a part of a 10 kb operon, which is negatively regulated by the yrzC gene product. FEMS Microbiol. Lett. 243: 51-58.
5. Sandoval, F.J., et al. 2005. An FMN hydrolase is fused to a riboflavin kinase homolog in plants. J. Biol. Chem. 280: 38337-38345.
6. Bertollo, C.M., et al. 2006. Characterization of the antinociceptive and anti-inflammatory activities of riboflavin in different experimental models. Eur. J. Pharmacol. 547: 184-191.
7. Ammelburg, M., et al. 2007. A CTP-dependent archaeal riboflavin kinase forms a bridge in the evolution of cradle-loop barrels. Structure 15: 1577-1590.

CHROMOSOMAL LOCATION

Genetic locus: Rfk (mouse) mapping to 19 B.

PRODUCT

Riboflavin kinase 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 Riboflavin kinase shRNA Plasmid (m): sc-62941-SH and Riboflavin kinase shRNA (m) Lentiviral Particles: sc-62941-V as alternate gene silencing products.

For independent verification of Riboflavin kinase (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-62941A, sc-62941B and sc-62941C.

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

Riboflavin kinase siRNA (m) is recommended for the inhibition of Riboflavin kinase 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

Riboflavin kinase (E-7): sc-398830 is recommended as a control antibody for monitoring of Riboflavin kinase 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 Riboflavin kinase gene expression knockdown using RT-PCR Primer: Riboflavin kinase (m)-PR: sc-62941-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.