SANTA CRUZ BIOTECHNOLOGY, INC.

YT521-B siRNA (h): sc-88938

BACKGROUND

YT521-B (YTH domain-containing protein 1), also known as YT521, is a 727 amino acid nuclear protein that localizes to the novel subnuclear structure of YT bodies and is the human homolog of the mouse gene, YTHDC1. Ubiquitously expressed, YT521-B may be part of a signal transduction pathway that influences splice site selection. YT521-B shuttles between the nucleus and cytosol, where it can be phosphorylated by c-Src or Fyn. Tyrosine phosphorylation by c-Abl causes dispersion of YT521-B from YT bodies to the nucleoplasm. Tyrosine phosphorylation also promotes sequestration of YT521-B in an insoluble nuclear form, which abolishes the ability of YT521-B to change alternative splice sites. YT521-B is considered to be a candidate for a role in a gene expression model of the pathogenesis of EDMD (Emery-Dreifuss muscular dystrophy), a type of muscular dystrophy primarily affecting voluntary muscles. YT521-B exists as two isoforms due to alternative splicing events.

REFERENCES

1. Imai, Y., et al. 1998. Cloning of a gene, YT521, for a novel RNA splicing-related protein induced by hypoxia/reoxygenation. Brain Res. Mol. Brain Res. 53: 33-40.
2. Hartmann, A.M., et al. 1999. The interaction and colocalization of Sam68 with the splicing-associated factor YT521-B in nuclear dots is regulated by the Src family kinase $p59^{Fyn}$. Mol. Biol. Cell 10: 3909-3926.
3. Nayler, O., et al. 2000. The ER repeat protein YT521-B localizes to a novel subnuclear compartment. J. Cell Biol. 150: 949-962.
4. Stoss, O., et al. 2001. The STAR/GSG family protein rSLM-2 regulates the selection of alternative splice sites. J. Biol. Chem. 276: 8665-8673.
5. Stoilov, P., et al. 2002. YTH: a new domain in nuclear proteins. Trends Biochem. Sci. 27: 495-497.
6. Wilkinson, F.L., et al. 2003. Emerin interacts *in vitro* with the splicing-associated factor, YT521-B. Eur. J. Biochem. 270: 2459-2466.

CHROMOSOMAL LOCATION

Genetic locus: YTHDC1 (human) mapping to 4q13.2.

PRODUCT

YT521-B 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 YT521-B shRNA Plasmid (h): sc-88938-SH and YT521-B shRNA (h) Lentiviral Particles: sc-88938-V as alternate gene silencing products.

For independent verification of YT521-B (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-88938A, sc-88938B and sc-88938C.

PROTOCOLS

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

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

YT521-B siRNA (h) is recommended for the inhibition of YT521-B 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

YT521-B (8): sc-136428 is recommended as a control antibody for monitoring of YT521-B 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 YT521-B gene expression knockdown using RT-PCR Primer: YT521-B (h)-PR: sc-88938-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.