SANTA CRUZ BIOTECHNOLOGY, INC.

BRUNOL4 siRNA (h): sc-72666

BACKGROUND

Members of the CELF (CUG-BP1- and ETR-3-like factor) family are RNA-binding proteins implicated in the regulation of pre-mRNA alternative splicing. The CELF family includes six members. CELF4, also designated BRUNOL4, mediates exon inclusion and/or exclusion in pre-mRNAs that are subject to tissue-specific and developmentally regulated alternative splicing. Specifically, BRUNOL4 activates exon 5 inclusion of cardiac isoforms of Troponin T-C during heart remodeling at the juvenile to adult transition, and promotes exclusion of both the smooth muscle (SM) and non-muscle (NM) exons in actinin pre-mRNAs. BRUNOL4 contains three RRM (RNA recognition motif) domains and binds to muscle-specific splicing enhancer (MSE) intronic sites flanking the alternative exon 5 of Troponin T-C pre-mRNA. BRUNOL4 is strongly expressed in cerebellum, hippocampus, amygdala, temporal and frontal cortex and frontal lobes. Disruption of the gene encoding BRUNOL4 results in idiopathic epilepsy, a common human disorder that leads to severe seizures.

REFERENCES

1. Ladd, A.N., et al. 2001. The CELF family of RNA binding proteins is implicated in cell-specific and developmentally regulated alternative splicing. Mol. Cell. Biol. 21: 1285-1296.
2. Meins, M., et al. 2002. Identification and characterization of murine BRUNOL4, a new member of the elav/bruno family. Cytogenet. Genome Res. 97: 254-260.
3. Gromak, N., et al. 2003. Antagonistic regulation of α-actinin alternative splicing by CELF proteins and polypyrimidine tract binding protein. RNA 9: 443-456.
4. Singh, G., et al. 2004. ETR-3 and CELF4 protein domains required for RNA binding and splicing activity *in vivo*. Nucleic Acids Res. 32: 1232-1241.
5. Ladd, A.N., et al. 2005. Cardiac tissue-specific repression of CELF activity disrupts alternative splicing and causes cardiomyopathy. Mol. Cell. Biol. 25: 6267-6278.

CHROMOSOMAL LOCATION

Genetic locus: CELF4 (human) mapping to 18q12.2.

PRODUCT

BRUNOL4 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 BRUNOL4 shRNA Plasmid (h): sc-72666-SH and BRUNOL4 shRNA (h) Lentiviral Particles: sc-72666-V as alternate gene silencing products.

For independent verification of BRUNOL4 (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-72666A, sc-72666B and sc-72666C.

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

BRUNOL4 siRNA (h) is recommended for the inhibition of BRUNOL4 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

BRUNOL4 (C-4): sc-398292 is recommended as a control antibody for monitoring of BRUNOL4 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 BRUNOL4 gene expression knockdown using RT-PCR Primer: BRUNOL4 (h)-PR: sc-72666-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.