SANTA CRUZ BIOTECHNOLOGY, INC.

HES2 siRNA (m): sc-37941

BACKGROUND

The *Drosophila* hairy and Enhancer of split genes encode basic helix-loop-helix (bHLH) transcriptional repressors that function in the Notch signaling pathway and control segmentation and neural development during embryogenesis. The mammalian homologues of *Drosophila* hairy and Enhancer of split are the HES gene family members, HES1-6, which also encode bHLH transcriptional repressors that regulate myogenesis and neurogenesis. The HES family members form a complex with TLE, the mammalian homologue of Groucho, and this interaction is mediated by the carboxy terminal WRPW motif of the HES proteins. The HES/TLE complex functions by directly binding to DNA, instead of interfering with activator proteins. Most HES family members, including HES1 and HES5, preferentially bind to the N box (CACNAG) as opposed to the E box (CANNTG). HES2 binds to both N and E box sites, while HES6 does not bind DNA. Rather, HES6 inhibits HES1 activity, thereby promoting transcription. HES1 and HES2 are expressed in a variety of adult and embryonic tissues. HES3 is expressed exclusively in cerebellar Purkinje cells, and HES5 is found solely in the nervous system. HES6 is produced in brain as well as in the limb buds of developing embryos.

REFERENCES

1. Sasai, Y., et al. 1992. Two mammalian helix-loop-helix factors structurally related to *Drosophila* Hairy and Enhancer of split. Genes Dev. 6: 2620-2634.
2. Akazawa, C., et al. 1992. Molecular characterization of a rat negative regulator with a basic helix-loop-helix structure predominantly expressed in the developing nervous system. J. Biol. Chem. 267: 21879-21885.
3. Ishibashi, M., et al. 1993. Molecular characterization of HES2, a mammalian helix-loop-helix factor structurally related to *Drosophila* Hairy and Enhancer of split. Eur. J. Biochem. 215: 645-652.
4. Takebayashi, K., et al. 1994. Structure, chromosomal locus and promoter analysis of the gene encoding the mouse helix-loop-helix factor HES1. Negative autoregulation through the multiple N box elements. J. Biol. Chem. 269: 5150-5156.
5. Fisher, A.L., et al. 1996. The WRPW motif of the hairy-related basic helix-loop-helix repressor proteins acts as a 4 amino-acid transcription repression and protein-protein interaction domain. Mol. Cell. Biol. 16: 2670-2677.

CHROMOSOMAL LOCATION

Genetic locus: Hes2 (mouse) mapping to 4 E2.

PRODUCT

HES2 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 HES2 shRNA Plasmid (m): sc-37941-SH and HES2 shRNA (m) Lentiviral Particles: sc-37941-V as alternate gene silencing products.

For independent verification of HES2 (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-37941A, sc-37941B and sc-37941C.

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

HES2 siRNA (m) is recommended for the inhibition of HES2 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

HES2 (H-8): sc-514711 is recommended as a control antibody for monitoring of HES2 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 HES2 gene expression knockdown using RT-PCR Primer: HES2 (m)-PR: sc-37941-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.