SANTA CRUZ BIOTECHNOLOGY, INC.

ENC1 siRNA (h): sc-91870

BACKGROUND

Proteolytic degradation by the ubiquitin (Ub) system is essential for normal cell cycle progression, cellular differentiation and stress responses. The E3 ubiquitin-protein ligase complex uses a substrate-specific adapter, ENC1 (ectoderm-neural cortex protein 1), to mediate ubiquitination. ENC1, also known as NRPB or PIG10, is a 589 amino acid Actin-binding protein that is involved in differentiation of neural crest cells and regulation of neuronal process formation. ENC1 is localized to the nuclear matrix and is highly expressed in adult brain and spinal cord tissues. Expression of ENC1 is up-regulated during neuronal differentiation. ENC1 may be regulated by the β-catenin/TCF pathway and is thought to play a role in histogenesis. ENC1 interacts with hypophosphorylated Rb (retinoblastoma-associated protein) to form a complex that contains CUL-3, Rbx1 and ENC1 which is essential for neuronal cell differentiation. ENC1 contains one BTB (POZ) domain and six Kelch repeats. The BTB domain is thought to be necessary for the protein-protein interactions involved in cytoskeletal organization and the Kelch repeats denote a conserved tertiary structure. ENC1 is highly expressed in brain tumors, suggesting a possible role in carcinogenesis.

REFERENCES

1. Polyak, K., et al. 1997. A model for p53-induced apoptosis. Nature 389: 300-305.
2. Hernandez, M.C., et al. 1998. Cloning of human ENC1 and evaluation of its expression and regulation in nervous system tumors. Exp. Cell Res. 242: 470-477.
3. Kim, T.A., et al. 1998. NRP/B, a novel nuclear matrix protein, associates with $p110^{RB}$ and is involved in neuronal differentiation. J. Cell Biol. 141: 553-566.
4. Hernandez, M.C., et al. 1999. Assignment of the ectodermal-neural cortex 1 gene (ENC1) to human chromosome band 5q13 by *in situ* hybridization. Cytogenet. Cell Genet. 87: 89-90.
5. Hernandez, M., et al. 2000. Assignment of the ectodermal-neural cortex 1 gene (Enc1) to mouse chromosome band 13D1 by fluorescence *in situ* hybridization. Cytogenet. Cell Genet. 89: 158-159.

CHROMOSOMAL LOCATION

Genetic locus: ENC1 (human) mapping to 5q13.3.

PRODUCT

ENC1 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 ENC1 shRNA Plasmid (h): sc-91870-SH and ENC1 shRNA (h) Lentiviral Particles: sc-91870-V as alternate gene silencing products.

For independent verification of ENC1 (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-91870A, sc-91870B and sc-91870C.

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

ENC1 siRNA (h) is recommended for the inhibition of ENC1 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

ENC1 (7a): sc-517590 is recommended as a control antibody for monitoring of ENC1 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) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) 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 ENC1 gene expression knockdown using RT-PCR Primer: ENC1 (h)-PR: sc-91870-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.