SANTA CRUZ BIOTECHNOLOGY, INC.

EWS siRNA (h): sc-35347

BACKGROUND

EWS is a nuclear RNA-binding protein. As a result of chromosome translocation, the EWS gene is fused to a variety of transcription factors, including ATF-1 in human neoplasias. In the Ewing family of tumors, the N-terminal domain of EWS is fused to the DNA-binding domain of various ETS transcription factors, including Fli-1, Erg, ETV1, E1AF and FEV. The EWS/Fli-1 chimeric protein acts as a more potent transcriptional activator than Fli-1 and can promote cell transformation. Two functional regions have been identified in EWS; an amino-terminal region (domain A), that has little transactivation activity but transforms efficiently when fused to Fli-1, and a distal region (domain B), which shows transactivation activity but transforms less efficiently when fused to Fli-1.

REFERENCES

1. Delattre, O., et al. 1992. Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. Nature 359: 162-165.
2. May, W.A., et al. 1993. The Ewing's sarcoma EWS/Fli-1 fusion gene encodes a more potent transcriptional activator and is a more powerful transforming gene than Fli-1. Mol. Cell. Biol. 13: 7393-7398.
3. Sorenson, P.H., et al. 1994. A second Ewing's sarcoma translocation, t(21;22), fuses the EWS gene to another ETS-family transcription factor, ERG. Nat. Genet. 6: 146-151.
4. Lessnick, S.L., et al. 1995. Multiple domains mediate transformation by the Ewing's sarcoma EWS/Fli-1 fusion gene. Oncogene 10: 423-431.

CHROMOSOMAL LOCATION

Genetic locus: EWSR1 (human) mapping to 22q12.2.

PRODUCT

EWS 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 EWS shRNA Plasmid (h): sc-35347-SH and EWS shRNA (h) Lentiviral Particles: sc-35347-V as alternate gene silencing products.

For independent verification of EWS (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-35347A, sc-35347B and sc-35347C.

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

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

EWS (G-5): sc-28327 is recommended as a control antibody for monitoring of EWS 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 EWS gene expression knockdown using RT-PCR Primer: EWS (h)-PR: sc-35347-PR (20 µl, 536 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Azuma, M., et al. 2007. Ewing sarcoma protein ewsr1 maintains mitotic integrity and proneural cell survival in the zebrafish embryo. PLoS ONE 2: e979.
2. Park, H., et al. 2014. Ewing sarcoma EWS protein regulates midzone formation by recruiting Aurora B kinase to the midzone. Cell Cycle 13: 2391-2399.
3. Verdile, V., et al. 2022. EWS splicing regulation contributes to balancing Foxp1 isoforms required for neuronal differentiation. Nucleic Acids Res. 50: 3362-3378.

RESEARCH USE

For research use only, not for use in diagnostic procedures.