

FUS/TLS siRNA (m): sc-40564

BACKGROUND

EWS and FUS/TLS are nuclear RNA-binding proteins. 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, ETV1 and FEV. The EWS/Fli-1 chimeric protein acts as a more potent transcriptional activator than Fli-1 and can promote cell transformation. In human myxoid liposarcomas and myeloid leukemias, chromosomal translocation results in the fusion of the N-terminal region of FUS/TLS with the open reading frame of CHOP. In normal cells, FUS/TLS binds to the DNA-binding domains of nuclear steroid receptors and is also present in subpopulations of TFIIID complexes, indicating a potential role for FUS/TLS in the processing of primary transcripts that are generated in response to hormone-induced transcription.

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. Crozat, A., et al. 1993. Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. *Nature* 363: 640-644.
4. Jeon, I.S., et al. 1995. A variant Ewing's sarcoma translocation (7;22) fuses the EWS gene to the ETS gene ETV1. *Oncogene* 10: 1229-1234.
5. Fujimura, Y., et al. 1996. The EWS-ATF-1 gene involved in malignant melanoma of soft parts with t(12;22) chromosome translocation, encodes a constitutive transcriptional activator. *Oncogene* 12: 159-167.
6. Peter, M., et al. 1997. A new member of the ETS family fused to EWS in Ewing tumors. *Oncogene* 14: 1159-1164.
7. Powers, C.A., et al. 1998. TLS (translocated-in-liposarcoma) is a high-affinity interactor for steroid, thyroid hormone, and retinoid receptors. *Mol. Endocrinol.* 12: 4-18.

CHROMOSOMAL LOCATION

Genetic locus: Fus (mouse) mapping to 7 F3.

PRODUCT

FUS/TLS 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see FUS/TLS shRNA Plasmid (m): sc-40564-SH and FUS/TLS shRNA (m) Lentiviral Particles: sc-40564-V as alternate gene silencing products.

For independent verification of FUS/TLS (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-40564A, sc-40564B and sc-40564C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

FUS/TLS siRNA (m) is recommended for the inhibition of FUS/TLS 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 μ M in 66 μ l. 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

FUS/TLS (4H11): sc-47711 is recommended as a control antibody for monitoring of FUS/TLS 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 FUS/TLS gene expression knockdown using RT-PCR Primer: FUS/TLS (m)-PR: sc-40564-PR (20 μ l, 576 bp). 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.