SANTA CRUZ BIOTECHNOLOGY, INC.

FAST-1/2 siRNA (m): sc-35363

BACKGROUND

Xenopus winged-helix factor, xFAST-1 (forkhead Activin signal transducer-1) is a transcription factor that forms a complex with the receptor-regulated Smad protein, Smad2, and directly binds to Activin response elements on DNA. The human homolog FAST-1 and the corresponding mouse homolog, designated FAST-2, share significant sequence homology with xFAST-1, including a conserved N-terminal forkhead domain that consists of 110 amino acid residues and is essential for binding DNA and regulating transcription in embryogenesis, in tumorigenesis and in the maintenance of differentiated cell states. FAST-1 and FAST-2 also contain a distinct C-terminal Smad interaction domain that is required for the association with various Smad proteins, including Smad2, Smad3 and Smad4. Expression of FAST-1 and FAST-2 is predominantly observed during early development, with lower levels detected in adult tissues. FAST-1 and FAST-2 mediated DNA binding is attenuated by both TFGβ and Activin, indicating that these FAST proteins mediate TFGβ induced signal transduction.

REFERENCES

1. Chen, X., et al. 1997. Smad4 and FAST-1 in the assembly of Activin-responsive factor. Nature 389: 85-89.
2. Labbe, E., et al. 1998. Smad2 and Smad3 positively and negatively regulate TGF β-dependent transcription through the forkhead DNA-binding protein FAST-2. Mol. Cell 2: 109-120.
3. Zhou, S., et al. 1998. Characterization of human FAST-1, a TGFβ and Activin signal transducer. Mol. Cell 2: 121-127.
4. Weisberg, E., et al. 1998. A mouse homologue of FAST-1 transduces TGFβ superfamily signals and is expressed during early embryogenesis. Mech. Dev. 79: 17-27.
5. Yeo, C.Y., et al. 1999. The role of FAST-1 and Smads in transcriptional regulation by Activin during early *Xenopus* embryogenesis. J. Biol. Chem. 274: 26584-26590.
6. Nagarajan, R.P., et al. 1999. Smad3 inhibits transforming growth factor-β and Activin signaling by competing with Smad4 for FAST-2 binding. J. Biol. Chem. 274: 31229-31235.

CHROMOSOMAL LOCATION

Genetic locus: Foxh1 (mouse) mapping to 15 D3.

PRODUCT

FAST-1/2 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 FAST-1/2 shRNA Plasmid (m): sc-35363-SH and FAST-1/2 shRNA (m) Lentiviral Particles: sc-35363-V as alternate gene silencing products.

For independent verification of FAST-1/2 (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-35363A, sc-35363B and sc-35363C.

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

FAST-1/2 siRNA (m) is recommended for the inhibition of FAST-1/2 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

FAST-1/2 (D-12): sc-377358 is recommended as a control antibody for monitoring of FAST-1/2 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 FAST-1/2 gene expression knockdown using RT-PCR Primer: FAST-1/2 (m)-PR: sc-35363-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.