SANTA CRUZ BIOTECHNOLOGY, INC.

fish siRNA (m): sc-35377

BACKGROUND

Fish, a potential Src substrate, is a broadly expressed adaptor protein containing five SH3 domains and a phox homology (PX) domain. The Src family of protein tyrosine kinases act in signal transduction pathways. Src kinases vary in expression but are strongly regulated *in vivo;* catalytic activity is repressed by interacting with the SH3 domain. In Src-transformed fibroblasts and in normal cells treated with certain growth factors fish is tyrosine-phosphorylated. Treatment of cells with cytochalasin D results in rapid tyrosine phosphorylation of fish, along with activation of Src. Fish is likely to be involved in tyrosine kinase signaling and may have a role in cytoskeletal changes.

REFERENCES

1. Bolen, J.B., et al. 1992. The Src family of tyrosine protein kinases in hemopoietic signal transduction. FASEB J. 6: 3403-3409.
2. Erpel, T. and Courtneidge, S.A. 1995. Src family protein tyrosine kinases and cellular signal transduction pathways. Curr. Opin. Cell Biol. 7: 176-182.
3. Superti-Furga, G. and Courtneidge, S.A. 1995. Structure-function relationships in Src family and related protein tyrosine kinases. Bioessays 17: 321-330.
4. Dikic, I., et al. 1996. A role for PYK2 and Src in linking G protein-coupled receptors with MAP kinase activation. Nature 383: 547-550.
5. Luttrell, L.M., et al. 1996. Role of c-Src tyrosine kinase in G protein-coupled receptor- and $G_{\beta\gamma}$ subunit-mediated activation of mitogen-activated protein kinases. J. Biol. Chem. 271: 19443-19450.
6. Brown, M.T. and Cooper, J.A. 1996. Regulation, substrates and functions of Src. Biochim. Biophys. Acta 1287: 121-149.
7. Lock, P., et al. 1998. A new method for isolating tyrosine kinase substrates used to identify fish, an SH3 and PX domain-containing protein, and Src substrate. EMBO J. 17: 4346-4357.

CHROMOSOMAL LOCATION

Genetic locus: Sh3pxd2a (mouse) mapping to 19 C3.

PRODUCT

fish 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 fish shRNA Plasmid (m): sc-35377-SH and fish shRNA (m) Lentiviral Particles: sc-35377-V as alternate gene silencing products.

For independent verification of fish (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-35377A, sc-35377B and sc-35377C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

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

fish (G-7): sc-376211 is recommended as a control antibody for monitoring of fish 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 fish gene expression knockdown using RT-PCR Primer: fish (m)-PR: sc-35377-PR (20 µl, 440 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Stylli, S.S., et al. 2009. Nck adaptor proteins link Tks5 to invadopodia Actin regulation and ECM degradation. J. Cell Sci. 122: 2727-2740.

RESEARCH USE

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