

## fish siRNA (h): sc-35376

### 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 hematopoietic 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.

### CHROMOSOMAL LOCATION

Genetic locus: SH3PXD2A (human) mapping to 10q24.33.

### PRODUCT

fish 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see fish shRNA Plasmid (h): sc-35376-SH and fish shRNA (h) Lentiviral Particles: sc-35376-V as alternate gene silencing products.

For independent verification of fish (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-35376A, sc-35376B and sc-35376C.

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

### APPLICATIONS

fish siRNA (h) is recommended for the inhibition of fish 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  $\mu$ M in 66  $\mu$ 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

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-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>TM</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> 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 (h)-PR: sc-35376-PR (20  $\mu$ l, 586 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. 2014. Expression of the adaptor protein Tks5 in human cancer: prognostic potential. *Oncol. Rep.* 32: 989-1002.
2. Moodley, S., et al. 2015. XB130/Tks5 scaffold protein interaction regulates Src-mediated cell proliferation and survival. *Mol. Biol. Cell* 26: 4492-4502.
3. Moodley, S., et al. 2016. Stimulus-dependent dissociation between XB130 and Tks5 scaffold proteins promotes airway epithelial cell migration. *Oncotarget* 7: 76437-76452.

### RESEARCH USE

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

### PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.