

# Sam 68 siRNA (h): sc-29476

## BACKGROUND

Sam 68 is a protein that is phosphorylated on tyrosine and functions as a substrate for Src family tyrosine kinases during mitosis. Sam 68 also associates with several SH2 and SH3 domain-containing signaling proteins, such as GRB2 and PLC  $\gamma$ 1. Originally cloned as Ras GAP-associated p62, further investigations have shown that Sam 68 and Ras GAP-associated p62 are not antigenically related, nor are they encoded by the same gene. Like Sam 68, the Sam 68-like mammalian proteins, SLM-1 and SLM-2, demonstrate RNA binding activity. Also like Sam 68, SLM-1 is tyrosine phosphorylated and functions as an adapter protein for signaling molecules, including GRB2, PLC  $\gamma$ 1, Fyn and Ras GAP. SLM-2 is not tyrosine phosphorylated, nor does it appear to associate with GRB2, PLC  $\gamma$ 1, Fyn or Ras GAP, indicating that SLM-2 may not be an adapter protein for these proteins.

## REFERENCES

1. Fumagalli, S., et al. 1994. A target for Src in mitosis. *Nature* 368: 871-874.
2. Maa, M.C., et al. 1994. A protein that is highly related to GTPase-activating protein-associated p62 complexes with PLC  $\gamma$ . *Mol. Cell. Biol.* 14: 5466-5473.
3. Richard, S., et al. 1995. Association of p62, a multifunctional SH2- and SH3-domain-binding protein, with Src family tyrosine kinases, GRB2, and PLC  $\gamma$ 1. *Mol. Cell. Biol.* 15: 186-197.
4. Lock, P., et al. 1996. The human p62 cDNA encodes Sam 68 and not the Ras GAP-associated p62 protein. *Cell* 84: 23-24.
5. Guitard, E., et al. 1998. Sam 68 is a Ras GAP-associated protein in mitosis. *Biochem. Biophys. Res. Commun.* 245: 562-566.

## CHROMOSOMAL LOCATION

Genetic locus: KHDRBS1 (human) mapping to 1p35.1.

## PRODUCT

Sam 68 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 Sam 68 shRNA Plasmid (h): sc-29476-SH and Sam 68 shRNA (h) Lentiviral Particles: sc-29476-V as alternate gene silencing products.

For independent verification of Sam 68 (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-29476A, sc-29476B and sc-29476C.

## 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

Sam 68 siRNA (h) is recommended for the inhibition of Sam 68 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

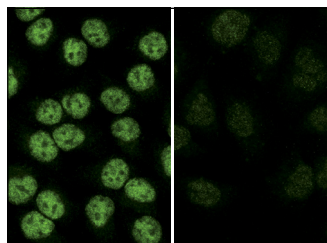
Sam 68 (H-4): sc-514468 is recommended as a control antibody for monitoring of Sam 68 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

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™ Molecular Weight Standards: sc-2035, UltraCruz® 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® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Sam 68 gene expression knockdown using RT-PCR Primer: Sam 68 (h)-PR: sc-29476-PR (20  $\mu$ l, 485 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## DATA



Sam 68 siRNA (h): sc-29476. Immunofluorescence staining of methanol-fixed, control HeLa (A) and Sam 68 siRNA silenced HeLa (B) cells showing diminished nuclear staining in the siRNA silenced cells. Cells probed with Sam 68 (C-20): sc-333.

## RESEARCH USE

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