

# ICAT siRNA (m): sc-45273

## BACKGROUND

ICAT interacts directly with  $\beta$ -catenin and interferes with the Wnt signaling pathway. Specifically, ICAT prevents the interaction of  $\beta$ -catenin with TCF-4 and inhibits  $\beta$ -catenin—TCF-4-mediated transactivation. The negative regulatory effect of ICAT on the Wnt signaling pathway appears to inhibit tumor cell proliferation. ICAT also induces G2 arrest followed by cell death in colorectal tumor cells. The ectopic induction of ICAT inhibits the expression of  $\beta$ 3 Tubulin and thus neuronal differentiation in embryonal carcinoma P19 cells. Structural characteristics of ICAT include a three-helix bundle and a C-terminal tail. The gene encoding human ICAT maps to chromosome 1p36.22.

## REFERENCES

1. Tago, K., et al. 2000. Inhibition of Wnt signaling by ICAT, a novel  $\beta$ -catenin-interacting protein. *Genes Dev.* 14: 1741-1749.
2. Sekiya, T., et al. 2002. Overexpression of ICAT induces G<sub>2</sub> arrest and cell death in tumor cell mutants for adenomatous polyposis coli,  $\beta$ -catenin, or Axin. *Cancer Res.* 62:3322-3326.
3. Graham, T.A., et al. 2002. The crystal structure of the  $\beta$ -catenin/ICAT complex reveals the inhibitory mechanism of ICAT. *Mol. Cell* 10: 563-571.
4. Reifemberger, J., et al. 2002. Molecular genetic analysis of malignant melanomas for aberrations of the Wnt signaling pathway genes CTNNB1, APC, ICAT and BTTC. *Int. J. Cancer* 100: 549-556.
5. Lyu, J., et al. 2003. Ectopic expression of Axin blocks neuronal differentiation of embryonic carcinoma P19 cells. *J. Biol. Chem.* 278: 13487-13495.

## CHROMOSOMAL LOCATION

Genetic locus: Ctnnbp1 (mouse) mapping to 4 E2.

## PRODUCT

ICAT 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ICAT shRNA Plasmid (m): sc-45273-SH and ICAT shRNA (m) Lentiviral Particles: sc-45273-V as alternate gene silencing products.

For independent verification of ICAT (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-45273A, sc-45273B and sc-45273C.

## 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

ICAT siRNA (m) is recommended for the inhibition of ICAT 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  $\mu$ 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

ICAT (5C6): sc-293489 is recommended as a control antibody for monitoring of ICAT 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 ICAT gene expression knockdown using RT-PCR Primer: ICAT (m)-PR: sc-45273-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Mu, J., et al. 2013. Functional implications of microRNA-215 in TGF $\beta$ 1-induced phenotypic transition of mesangial cells by targeting CTNNBIP1. *PLoS ONE* 8: e58622.

## 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.