SANTA CRUZ BIOTECHNOLOGY, INC.

Ski siRNA (h): sc-38366

BACKGROUND

The Ski family of oncogenes includes Ski and Sno (Ski-related novel gene, or Ski-like). Three isoforms of human Sno (SnoN, SnoA and SnoI) and 2 isoforms in mouse (SnoN and SnoN2, also designated sno-dE3) are produced by alternative splicing of the SKIL gene. Ski family members are nuclear proteins that form homodimers and heterodimers, bind to DNA and function as transcriptional activators and repressors. These proteins consist of five tandem repeats in the C-terminal domain and two leucine zipper motifs that are responsible for efficient DNA binding, trimerization and cellular transformation. The Ski proteins regulate TGFβ induced gene-specific transcriptional activation by effectively repressing Smad activity and, thereby, inhibit TGFβ induced cell growth and extracellular matrix production. The amino terminus of Ski and SnoN preferentially associates with the MH2 domain of Smad2 and Smad4 of the Smad family of transcription factors, where they then recruit the transcriptional co-repressor protein N-CoR to the complex to inhibit transcription. Alternatively, Ski proteins are negatively regulated by various Smad proteins, as TGFβ induces Smad3 accumulation in the nucleus, where it is then responsible for inducing the rapid degradation of SnoN and faciliating TGFβ signaling pathways and Smad-activated gene transcription.

REFERENCES

1. Nomura, N., et al. 1989. Isolation of human cDNA clones of Ski and the Ski-related gene, Sno. Nucleic Acids Res. 17: 5489-5500.
2. Pearson-White, S. 1993. SnoI, a novel alternatively spliced isoform of the Ski protooncogene homolog, Sno. Nucleic Acids Res. 21: 4632-4638.
3. Nagase, T., et al. 1993. Complex formation between proteins encoded by the Ski gene family. J. Biol. Chem. 268: 13710-13716.
4. Heyman, H.C. and Stavnezer, E. 1994. A carboxyl-terminal region of the Ski oncoprotein mediates homodimerization as well as heterodimerization with the related protein SnoN. J. Biol. Chem. 269: 26996-27003.
5. Mimura, N., et al. 1996. A transient increase of SnoN transcript by growth arrest upon serum deprivation and cell-to-cell contact. FEBS Lett. 397: 253-259.
6. Stroschein, S.L., et al. 1999. Negative feedback regulation of TGF-β signaling by the SnoN oncoprotein. Science 286: 771-774.

CHROMOSOMAL LOCATION

Genetic locus: SKI (human) mapping to 1p36.33.

PRODUCT

Ski 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Ski shRNA Plasmid (h): sc-38366-SH and Ski shRNA (h) Lentiviral Particles: sc-38366-V as alternate gene silencing products.

For independent verification of Ski (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-38366A, sc-38366B and sc-38366C.

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

Ski siRNA (h) is recommended for the inhibition of Ski 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 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

Ski (G8): sc-33693 is recommended as a control antibody for monitoring of Ski 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 Ski gene expression knockdown using RT-PCR Primer: Ski (h)-PR: sc-38366-PR (20 µl, 448 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Levati, L., et al. 2011. MicroRNA-155 targets the SKI gene in human melanoma cell lines. Pigment Cell Melanoma Res. 24: 538-550.

RESEARCH USE

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