

ASC-1 siRNA (h): sc-39159

BACKGROUND

Activating signal cointegrator-1 (ASC-1, originally identified as TRIP4) is a transcriptional coactivator of nuclear receptors that associates with specific components of the RNA polymerase II complex and binds the basal transcription factors TBP and TFIIA. ASC-1 functions with the transcription integrators SRC-1 and CBP/p300 through its zinc-finger motif and is dependent on their ligand-dependent transactivation domain, AF2. Endogenous ASC-1 in HeLa cells is predominantly a nuclear protein. Under conditions of serum starvation, ASC-1 localizes to the cytoplasm. However, when serum starved in the presence of ligand or coexpressed CBP or SRC-1, ASC-1 remains in the nucleus. This behavior of ASC-1 suggests that it may play an important role in establishing distinct co-activator complexes under different cellular conditions.

REFERENCES

1. Lee, J.W., et al. 1995. Two classes of proteins dependent on either the presence or absence of thyroid hormone for interaction with the thyroid hormone receptor. *Mol. Endocrinol.* 9: 243-254.
2. Mangelsdorf, D.J., et al. 1995. The nuclear receptor superfamily: the second decade. *Cell* 83: 835-839.
3. Yao, T.P., et al. 1996. The nuclear hormone receptor coactivator SRC-1 is a specific target of p300. *Proc. Natl. Acad. Sci. USA* 93: 10626-10631.
4. Shibata, H., et al. 1997. Role of co-activators and co-repressors in the mechanism of steroid/thyroid receptor action. *Recent Prog. Horm. Res.* 52: 141-164.
5. Kim, H.J., et al. 1999. Activating signal cointegrator 1, a novel transcription coactivator of nuclear receptors, and its cytosolic localization under conditions of serum deprivation. *Mol. Cell. Biol.* 19: 6323-6332.

CHROMOSOMAL LOCATION

Genetic locus: TRIP4 (human) mapping to 15q22.31.

PRODUCT

ASC-1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ASC-1 shRNA Plasmid (h): sc-39159-SH and ASC-1 shRNA (h) Lentiviral Particles: sc-39159-V as alternate gene silencing products.

For independent verification of ASC-1 (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-39159A, sc-39159B and sc-39159C.

RESEARCH USE

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

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

APPLICATIONS

ASC-1 siRNA (h) is recommended for the inhibition of ASC-1 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 μ M in 66 μ 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

ASC-1 (F-8): sc-365202 is recommended as a control antibody for monitoring of ASC-1 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 ASC-1 gene expression knockdown using RT-PCR Primer: ASC-1 (h)-PR: sc-39159-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.