

DET1 siRNA (h): sc-89981

BACKGROUND

DET1 (de-etiolated homolog 1), is a 550 amino acid highly conserved nuclear protein that is expressed in the ovary, some lymphoid organs and resting leukocytes. DET1 is a component of the E3 ubiquitin ligase Doublecortin DET1-COP1 complex, which is required for ubiquitination and degradation of proto-oncogenic transcription factor c-Jun. DET1 enhances the activity of several members of the UBC (Ub-conjugating enzyme) family and modulates Cul-4A functions. DET1 is involved in multiple plant developmental processes and acts as an essential negative regulator of photomorphogenesis.

REFERENCES

1. Pepper, A., et al. 1994. DET1, a negative regulator of light-mediated development and gene expression in arabidopsis, encodes a novel nuclear-localized protein. *Cell* 78: 109-116.
2. Benvenuto, G., et al. 2002. The photomorphogenesis regulator DET1 binds the amino-terminal tail of histone H2B in a nucleosome context. *Curr. Biol.* 12: 1529-1534.
3. Davuluri, G.R., et al. 2004. Manipulation of DET1 expression in tomato results in photomorphogenic phenotypes caused by post-transcriptional gene silencing. *Plant J.* 40: 344-354.
4. Wertz, I.E., et al. 2004. Human De-etiolated-1 regulates c-Jun by assembling a CUL4A ubiquitin ligase. *Science* 303: 1371-1374.
5. Al Khateeb, W.M. and Schroeder, D.F. 2007. DDB2, DDB1A and DET1 exhibit complex interactions during *Arabidopsis* development. *Genetics* 176: 231-242.
6. Pick, E., et al. 2007. Mammalian DET1 regulates Cul4A activity and forms stable complexes with E2 ubiquitin-conjugating enzymes. *Mol. Cell. Biol.* 27: 4708-4719.
7. Anzi, S., et al. 2008. Transcriptional repression of c-Jun's E3 ubiquitin ligases contributes to c-Jun induction by UV. *Cell. Signal.* 20: 862-871.
8. Zhang, Y., et al. 2008. *Arabidopsis* DDB1-CUL4 ASSOCIATED FACTOR1 forms a nuclear E3 ubiquitin ligase with DDB1 and CUL4 that is involved in multiple plant developmental processes. *Plant Cell* 20: 1437-1455.

CHROMOSOMAL LOCATION

Genetic locus: DET1 (human) mapping to 15q25.3.

PRODUCT

DET1 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 DET1 shRNA Plasmid (h): sc-89981-SH and DET1 shRNA (h) Lentiviral Particles: sc-89981-V as alternate gene silencing products.

For independent verification of DET1 (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-89981A, sc-89981B and sc-89981C.

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

DET1 siRNA (h) is recommended for the inhibition of DET1 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

DET1 (F-2): sc-514348 is recommended as a control antibody for monitoring of DET1 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 DET1 gene expression knockdown using RT-PCR Primer: DET1 (h)-PR: sc-89981-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.