

## Evi-1 siRNA (h): sc-37873

### BACKGROUND

The Evi-1 proto-oncogene contains two zinc finger domains, the second of which is essential for transactivation of the c-Fos promoter and for AP-1 activation. The first zinc finger domain binds to Smad3, suppressing its activity and inhibiting TGF $\beta$  signaling. The t(3;21) (q26;q22) chromosomal translocation produces a chimeric transcription factor, AML-1/Evi-1, that appears to suppress the transactivation of AML-1, which is a stimulator of myeloid cell differentiation. Inappropriate Evi-1 gene expression in hematopoietic cells has been shown to be associated with acute myelogenous leukemia (AML) and myelodysplastic syndromes.

### REFERENCES

1. Kreider, B.L., et al. 1993. Loss of erythropoietin responsiveness in erythroid progenitors due to expression of the Evi-1 myeloid-transforming gene. *Proc. Natl. Acad. Sci. USA* 90: 6454-6458.
2. Tanaka, T., et al. 1994. Evi-1 raises AP-1 activity and stimulates c-fos promoter transactivation with dependence on the second zinc finger domain. *J. Biol. Chem.* 269: 24020-24026.
3. Tanaka, T., et al. 1995. Dual functions of the AML1/Evi-1 chimeric protein in the mechanism of leukemogenesis in t(3;21) leukemias. *Mol. Cell. Biol.* 15: 2383-2392.
4. Ogawa, S., et al. 1996. Abnormal expression of Evi-1 gene in human leukemias. *Hum. Cell* 9: 323-332.
5. Kurokawa, M., et al. 1998. The t(3;21) fusion product, AML1/Evi-1, interacts with Smad3 and blocks transforming growth factor- $\beta$ -mediated growth inhibition of myeloid cells. *Blood* 92: 4003-4012.
6. Kurokawa, M., et al. 1998. The oncoprotein Evi-1 represses TGF- $\beta$  signalling by inhibiting Smad3. *Nature* 394: 92-96.

### CHROMOSOMAL LOCATION

Genetic locus: MECOM (human) mapping to 3q26.2.

### PRODUCT

Evi-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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Evi-1 shRNA Plasmid (h): sc-37873-SH and Evi-1 shRNA (h) Lentiviral Particles: sc-37873-V as alternate gene silencing products.

For independent verification of Evi-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-37873A, sc-37873B and sc-37873C.

### 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.

### 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

Evi-1 siRNA (h) is recommended for the inhibition of Evi-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  $\mu$ 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

Evi-1 (H-8): sc-515456 is recommended as a control antibody for monitoring of Evi-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 $\kappa$  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 Evi-1 gene expression knockdown using RT-PCR Primer: Evi-1 (h)-PR: sc-37873-PR (20  $\mu$ l, 492 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.