

EAF1 siRNA (h): sc-62249

BACKGROUND

The ELL family of RNA Pol II elongation factors function to activate transcript elongation by inhibiting the transient pausing of RNA Pol II. ELL associated factors (EAF) 1 and 2 (EAF1 and EAF2) directly interact with the ELL family members, ELL and ELL2, functioning as transcriptional activators of their elongation activities. More specifically, EAF1 and EAF2 can form a complex with ELL that targets the ternary elongation complex of RNA Pol II, stimulating the rate of elongation. In addition, EAF1 and EAF2 are important for the stability of the NuA4 histone acetyltransferase complex which transcriptionally activates certain genes by acetylation of Histones H4 and H2A. EAF1 is an ubiquitously expressed member of the EAF family that colocalizes with ELL to the the Cajal bodies and nuclear speckles. It contains a C-terminal region rich in aspartic acid, glutamic acid and serine residues.

REFERENCES

1. Luo, R.T., et al. 2001. The elongation domain of ELL is dispensable but its ELL-associated factor 1 interaction domain is essential for MLL-ELL-induced leukemogenesis. *Mol. Cell. Biol.* 21: 5678-5687.
2. Simone, F., et al. 2001. EAF1, a novel ELL-associated factor that is delocalized by expression of the MLL-ELL fusion protein. *Blood* 98: 201-209.
3. Li, M., et al. 2003. Expression of murine ELL-associated factor 2 (EAF2) is developmentally regulated. *Dev. Dyn.* 228: 273-280.
4. Polak, P.E., et al. 2003. ELL and EAF1 are Cajal body components that are disrupted in MLL-ELL leukemia. *Mol. Biol. Cell* 14: 1517-1528.
5. Simone, F., et al. 2003. ELL-associated factor 2 (EAF2), a functional homolog of EAF1 with alternative ELL binding properties. *Blood* 101: 2355-2362.

CHROMOSOMAL LOCATION

Genetic locus: EAF1 (human) mapping to 3p25.1.

PRODUCT

EAF1 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 EAF1 shRNA Plasmid (h): sc-62249-SH and EAF1 shRNA (h) Lentiviral Particles: sc-62249-V as alternate gene silencing products.

For independent verification of EAF1 (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-62249A, sc-62249B and sc-62249C.

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

EAF1 siRNA (h) is recommended for the inhibition of EAF1 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

EAF1 (A-10): sc-373832 is recommended as a control antibody for monitoring of EAF1 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 EAF1 gene expression knockdown using RT-PCR Primer: EAF1 (h)-PR: sc-62249-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.