SANTA CRUZ BIOTECHNOLOGY, INC.

Ref-1 siRNA (r): sc-72399

BACKGROUND

The role of transcription factors in the regulation of gene expression is well established. Although the activity of these factors can be regulated by phosphorylation, evidence has indicated regulation of DNA binding mediated by changes in reduction-oxidation (redox) status. Mutational analysis has identified a single conserved cysteine residue mapping within the DNA binding domains of Fos and Jun. Chemical oxidation or modification of this cysteine residue inhibits the DNA binding activity of Fos and Jun. A similar mode of regulation has been recently proposed for other nuclear transcription factors. Oxidation is reversible by these compounds or by a cellular redox/DNA repair protein identified originally as Ref-1 (redox factor 1). Ref-1 is identical to a previously characterized DNA repair enzyme designated HAP1, APE or APEX.

REFERENCES

1. Abate, C., et al. 1990. Redox regulation of Fos and Jun DNA binding activity *in vitro*. Science 249: 1157-1161.
2. Boyle, W.J., et al. 1991. Activation of PKC decreases phosphorylation of c-Jun at sites that only regulate its DNA binding activity. Cell 64: 573-584.
3. Hunter, T., et al. 1992. The regulation of transcription by phosphorylation. Cell 70: 375-387.
4. Xanthoudakis, S., et al. 1992. Identification and characterization of Ref-1, a nuclear protein that facilitates AP-1 DNA-binding activity. EMBO J. 11: 653-665.
5. Xanthoudakis, S., et al. 1992. Redox activation of Fos-Jun DNA binding activity is mediated by a DNA repair enzyme. EMBO J. 11: 3323-3335.
6. Guehmann, S., et al. 1992. Reduction of a conserved Cys is essential for Myb DNA-binding. Nucleic Acids Res. 20: 2279-2286.
7. Walker, L.J., et al. 1993. Identification of residues in the human DNA repair enzyme HAP1 (Ref-1) that are essential for redox regulation of Jun DNA binding. Mol. Cell. Biol. 13: 5370-5376.
8. Xanthoudakis, S., et al. 1994. The redox and DNA-repair activities of Ref-1 are encoded by nonoverlapping domains. Proc. Natl. Acad. Sci. USA 91: 23-27.

CHROMOSOMAL LOCATION

Genetic locus: Apex1 (rat) mapping to 15.

PRODUCT

Ref-1 siRNA (r) 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 Ref-1 shRNA Plasmid (r): sc-72399-SH and Ref-1 shRNA (r) Lentiviral Particles: sc-72399-V as alternate gene silencing products.

For independent verification of Ref-1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-72399A, sc-72399B and sc-72399C.

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

Ref-1 siRNA (r) is recommended for the inhibition of Ref-1 expression in rat 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

Ref-1 (C-4): sc-17774 is recommended as a control antibody for monitoring of Ref-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-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 Ref-1 gene expression knockdown using RT-PCR Primer: Ref-1 (r)-PR: sc-72399-PR (20 µl). 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.