SANTA CRUZ BIOTECHNOLOGY, INC.

Mad 1 siRNA (m): sc-38074

BACKGROUND

It is now well established that the nature and relative abundance of individual subunits of different classes of transcription factors can positively or negatively regulate levels of gene expression. Myc proteins homodimerize and bind DNA poorly, if at all, at physiological levels. Max is a nuclear localized bHLH-Zip protein initially identified by screening a B cell expression library with the bHLH-Zip region of c-Myc. Max homodimers and the Myc-Max heterodimers bind the sequence CACGTG; however the binding of the heterodimeric complex is stronger than the Max homodimer. In contrast to Myc which is highly regulated during progression through the cell cycle, Max is highly stable and is much more abundant than Myc. Two members of the bHLH-Zip protein family, designated Mad and Mxi 1 homodimerize poorly but form heterodimeric complexes with Max that have opposing functions to Myc-Max heterodimers with respect to regulation of gene expression.

REFERENCES

1. Jones, N. 1990. Transcriptional regulation by dimerization: two sides to an incestuous relationship. Cell 61: 9-11.
2. Dang, C.V., et al. 1991. Intracellular leucine zipper interactions suggest c-Myc hetero-oligomerization. Mol. Cell. Biol. 11: 954-962.
3. Blackwood, E.M., et al. 1991. Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. Science 251: 1211-1217.
4. Prendergast, G.C., et al. 1991. Association of Myn, the murine homolog of Max, with c-Myc stimulates methylation-sensitive DNA binding and Ras cotransformation. Cell 65: 395-407.
5. Mukherjee, B., et al. 1992. Myc family oncoproteins function through a common pathway to transform normal cells in culture: cross-interference by Max and transacting dominant mutants. Genes Dev. 6: 1480-1492.
6. Ayer, D.E., et al. 1993. Mad: a heterodimeric partner for Max that antagonizes Myc transcriptional activity. Cell 72: 211-222.
7. Zervos, A.S., et al. 1993. Mxi1, a protein that specifically interacts with Max to bind Myc-Max recognition sites. Cell 72: 223-232.
8. Amati, B., et al. 1993. Oncogenic activity of the c-Myc protein requires dimerization with Max. Cell 72: 233-245.

CHROMOSOMAL LOCATION

Genetic locus: Mad (mouse) mapping to 6 D1.

PRODUCT

Mad 1 siRNA (m) 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 Mad 1 shRNA Plasmid (m): sc-38074-SH and Mad 1 shRNA (m) Lentiviral Particles: sc-38074-V as alternate gene silencing products.

For independent verification of Mad 1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-38074A, sc-38074B and sc-38074C.

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

Mad 1 siRNA (m) is recommended for the inhibition of Mad 1 expression in mouse 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

Mad 1 (F-1): sc-8012 is recommended as a control antibody for monitoring of Mad 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-IgGλ BP-HRP: sc-516132 or m-IgGλ BP-HRP (Cruz Marker): sc-516132-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-IgGλ BP-FITC: sc-516185 or m-IgGλ BP-PE: sc-516186 (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 Mad 1 gene expression knockdown using RT-PCR Primer: Mad 1 (m)-PR: sc-38074-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.