

Mad 3 siRNA (h): sc-38075

BACKGROUND

It is now well established that Myc regulation of cell proliferation and differentiation involves a family of related transcription factors. One such factor, Max, is an obligate heterodimeric partner for Myc and can also form heterodimers with at least four related proteins designated Mad 1, Mxi1 (i.e., Mad 2), Mad 3 and Mad 4. Like Mad 1 and Mxi1, association of Mad 3 and Mad 4 with Max results in transcriptional repression. Both Myc and the Mad proteins have short half-lives and their synthesis is tightly regulated while Max expression is constitutive and relatively stable. Two related mammalian cDNAs have been identified and shown to encode Mad-binding proteins. Both possess sequence homology with the yeast transcription repressor Sin3 including four conserved paired amphipathic helix (PAH) domains. mSin3A and mSin3B specifically interact with the Mad proteins via their second paired amphipathic helix domain (PAH2). It has been suggested that Mad-Max heterodimers repress transcription by tethering mSin3 to DNA as corepressors.

REFERENCES

1. 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.
2. Kretzner, L., et al. 1992. The Myc and Max proteins possess distinct transcriptional activities. *Nature* 359: 426-429.
3. Ayer, D.E., et al. 1993. Mad: a heterodimeric partner for Max that antagonizes Myc transcriptional activity. *Cell* 72: 211-222.
4. Amati, B., et al. 1993. The c-Myc protein induces cell cycle progression and apoptosis through dimerization with Max. *EMBO J.* 12: 5083-5087.
5. Ayer, D.E., et al. 1995. Mad-Max transcriptional repression is mediated by ternary complex formation with mammalian homologs of yeast repressor Sin3. *Cell* 80: 767-776.
6. Schrelber-Agus, N., et al. 1995. An amino-terminal domain of Mxi1 mediates anti-Myc oncogenic activity and interacts with a homolog of the yeast transcriptional repressor SIN3. *Cell* 80: 777-786.
7. Hurlin, P.J., et al. 1995. Mad 3 and Mad 4: novel Max-interacting transcriptional repressors that suppress c-Myc dependent transformation and are expressed during neural and epidermal differentiation. *EMBO J.* 14: 5646-5659.
8. Burton, J.L., et al. 2007. Mad3p, a pseudosubstrate inhibitor of APC^{Cdc20} in the spindle assembly checkpoint. *Genes Dev.* 21: 655-667.
9. Larsen, N.A., et al. 2007. Structural analysis of Bub3 interactions in the mitotic spindle checkpoint. *Proc. Natl. Acad. Sci. USA* 104: 1201-1206.

CHROMOSOMAL LOCATION

Genetic locus: MXD3 (human) mapping to 5q35.3.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

PRODUCT

Mad 3 siRNA (h) is a pool of 2 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 Mad 3 shRNA Plasmid (h): sc-38075-SH and Mad 3 shRNA (h) Lentiviral Particles: sc-38075-V as alternate gene silencing products.

For independent verification of Mad 3 (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-38075A and sc-38075B.

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

Mad 3 siRNA (h) is recommended for the inhibition of Mad 3 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Mad 3 gene expression knockdown using RT-PCR Primer: Mad 3 (h)-PR: sc-38075-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.