SANTA CRUZ BIOTECHNOLOGY, INC.

Makorin-1 siRNA (h): sc-62584

BACKGROUND

The Makorins are a family of proteins containing two to four C3H zinc fingers that may confer RNA-binding. In addition, they contain a C3HC4 RING zinc finger that allows them to function as E3 ubiquitin ligases. Makorin-1, also designated RING finger protein 61, is a ubiquitously expressed member of the Makorin family that mediates the ubiquitination of human TERT. The overexpression of Makorin-1 produces a variety of effects. In telomerase-positive cells, Makorin-1 overexpression promotes TERT degradation and reduces the activity of telomerase, leading to a reduction in telomere length. Overexpression of Makorin-1 also inhibits the transcriptional activities of c-Jun, the nuclear receptors, the androgen receptor and the retinoic acid receptors. The inhibitory activity of Makorin-1 requires both the amino- and carboxy-termini but not functioning ubiquitin ligase activity. The less common function of Makorin-1 as an activator also requires both the amino- and carboxy termini.

REFERENCES

1. Gray, T.A., Azama, K., Whitmore, K., Min, A., Abe, S. and Nicholls, R.D. 2001. Phylogenetic conservation of the Makorin-2 gene, encoding a multiple zinc-finger protein, antisense to the RAF1 proto-oncogene. Genomics 77: 119-126.
2. Kim, J.H., Park, S.M., Kang, M.R., Oh, S.Y., Lee, T.H., Muller, M.T. and Chung, I.K. 2005. Ubiquitin ligase Makorin-1 modulates telomere length homeostasis through a proteolysis of hTERT. Genes Dev. 19: 776-781.
3. Gray, T.A., Wilson, A., Fortin, P.J. and Nicholls, R.D. 2006. The putatively functional Makorin-1-p1 pseudogene is neither expressed nor imprinted, nor does it regulate its source gene in *trans*. Proc. Natl. Acad. Sci. USA 103: 12039-12044.
4. Omwancha, J., Zhou, X.F., Chen, S.Y., Baslan, T., Fisher, C.J., Zheng, Z., Cai, C. and Shemshedini, L. 2006. Makorin RING finger protein 1 (Makorin-1) has negative and positive effects on RNA polymerase II-dependent transcription. Endocrine 29: 363-373.

CHROMOSOMAL LOCATION

Genetic locus: MKRN1 (human) mapping to 7q34.

PRODUCT

Makorin-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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Makorin-1 shRNA Plasmid (h): sc-62584-SH and Makorin-1 shRNA (h) Lentiviral Particles: sc-62584-V as alternate gene silencing products.

For independent verification of Makorin-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-62584A, sc-62584B and sc-62584C.

PROTOCOLS

See our web site at 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 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

Makorin-1 siRNA (h) is recommended for the inhibition of Makorin-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 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

Makorin-1 (C-8): sc-515815 is recommended as a control antibody for monitoring of Makorin-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 Makorin-1 gene expression knockdown using RT-PCR Primer: Makorin-1 (h)-PR: sc-62584-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.