SANTA CRUZ BIOTECHNOLOGY, INC.

JMY siRNA (m): sc-35725

BACKGROUND

p300 and CBP (CREB-binding proteins) function as coactivators for various transcription factors, including p53. As cofactors, p300 and CBP possesses intrinsic acetyltransferase activity which may allow p300/CBP proteins to regulate transcription through direct acetylation and thereafter, enhance DNA binding activity. JMY is a nuclear cofactor for p300 that cooperatively enhances p53 activation in response to cellular stress. The p53 protein requires p300/CBP coactivator proteins in order to transcriptionally activate target genes. When p53 is activated, p300 component of the coactivator protein complexes associate with JMY and potentiate p53-dependent transcription and apoptosis. p53 acts as a sequence-specific transcription factor and upon stimulation, induces transcription of genes involved in growth arrest, including the waf1/cip1, bax, mdm2, and gadd45 genes. Disruption of p300 and JMY complexes inhibits p53-induced transcription of bax and blocks apoptosis. Due to alternative splicing, several isoforms of JMY are produced, and these various isoforms have different influencing affects on p53 activation, with some isoforms markedly enhancing p53 responses compared to the other splicing variants.

REFERENCES

1. Lill, N.L., et al. 1997. Binding and modulation of p53 by p300/CBP coactivators. Nature 387: 823-827.
2. Snowden, A.W., et al. 1998. Cell cycle regulation of the transcriptional coactivators p300 and CREB binding protein. Biochem. Pharmacol. 55: 1947-1954.
3. Thomas, A., et al. 1998. Suppression of the p300-dependent mdm2 negative-feedback loop induces the p53 apoptotic function. Genes Dev. 12: 1975-1985.
4. Liu, L., et al. 1999. p53 sites acetylated *in vitro* by PCAF and p300 are acetylated *in vivo* in response to DNA damage. Mol. Cell. Biol. 19: 1202-1209.
5. Shikama, N., et al. 1999. A novel cofactor for p300 that regulates the p53 response. Mol. Cell 4: 365-376.
6. Yuan, Z.M., et al. 1999. Role for p300 in stabilization of p53 in the response to DNA damage. J. Biol. Chem. 274: 1883-1886.

CHROMOSOMAL LOCATION

Genetic locus: Jmy (mouse) mapping to 13 C3.

PRODUCT

JMY 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 JMY shRNA Plasmid (m): sc-35725-SH and JMY shRNA (m) Lentiviral Particles: sc-35725-V as alternate gene silencing products.

For independent verification of JMY (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-35725A, sc-35725B and sc-35725C.

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

JMY siRNA (m) is recommended for the inhibition of JMY 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

JMY (G-11): sc-166030 is recommended as a control antibody for monitoring of JMY 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 JMY gene expression knockdown using RT-PCR Primer: JMY (m)-PR: sc-35725-PR (20 µl, 594 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Firat-Karalar, E.N., et al. 2011. The Actin nucleation factor JMY is a negative regulator of neuritogenesis. Mol. Biol. Cell 22: 4563-4574.

RESEARCH USE

For research use only, not for use in diagnostic procedures.