SANTA CRUZ BIOTECHNOLOGY, INC.

53BP1 siRNA (m): sc-37456

BACKGROUND

The p53 binding proteins 53BP1 and 53BP2 (Bbp) bind to the central DNA-binding domain of wildtype p53, but do not bind mutant p53. The central DNA-binding domain of p53 is required for site-specific DNA binding and is frequently mutated in malignant tumors. Binding of 53BP1 to the L3 loop of p53 and of 53BP2 to the L2 loop of p53 confirms that the loop is dependent on p53 conformation. Site-specific binding also suggests that 53BP1 and 53BP2 are involved in p53-mediated tumor suppression. 53BP1 was isolated from H258 cells and is expressed in Jurkat cells in both the cytoplasm and the nucleus. The N-terminus of 53BP2 is localized to the cytoplasm, while the C-terminus might be localized in the nucleus. 53BP1 promotes cell proliferation by binding to p202, whereas 53BP2 induces cell death by binding to Bcl2 and NFκB p65.

REFERENCES

1. Iwabuchi, K., et al. 1994. Two cellular proteins that bind to wildtype but not mutant p53. Proc. Natl. Acad. Sci. USA 91: 6098-6102.
2. Gorina, S. and Pavletich, N.P. 1996. Structure of the p53 tumor suppressor bound to the ankyrin and SH3 domains of 53BP2. Science 274: 1001-1005.
3. Naumovski, L. and Cleary, M.L. 1996. The p53-binding protein 53BP2 also interacts with Bcl12 and impedes cell cycle progression at G_2/M. Mol. Cell. Biol. 16: 3884-3892.
4. Bansidhar, D., et al. 1996. p202, an interferon-inducible modulator of transcription, inhibits transcriptional activation by the p53 tumor suppressor protein and a segment from the p53-binding protein 1 that binds to p202 overcomes this inhibition. J. Biol. Chem. 271: 27544-27555.
5. Iwabuchi, K., et al. 1998. Stimulation of p53-mediated transcriptional activation by the p53-binding proteins, 53BP1 and 53BP2. J. Biol. Chem. 273: 26061-26068.
6. Yang, J.P., et al. 1999. NFκB subunit p65 binds to 53BP2 and inhibits cell death induced by 53BP2. Oncogene 18: 5177-5186.

CHROMOSOMAL LOCATION

Genetic locus: Trp53bp1 (mouse) mapping to 2 E5.

PRODUCT

53BP1 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 53BP1 shRNA Plasmid (m): sc-37456-SH and 53BP1 shRNA (m) Lentiviral Particles: sc-37456-V as alternate gene silencing products.

For independent verification of 53BP1 (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-37456A, sc-37456B and sc-37456C.

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

53BP1 siRNA (m) is recommended for the inhibition of 53BP1 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

53BP1 (E-10): sc-515841 is recommended as a control antibody for monitoring of 53BP1 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 53BP1 gene expression knockdown using RT-PCR Primer: 53BP1 (m)-PR: sc-37456-PR (20 µl, 511 bp). 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.