SANTA CRUZ BIOTECHNOLOGY, INC.

ASPP1 siRNA (h): sc-60214

BACKGROUND

ASPP proteins interact with p53 and are responsible for enhancing p53-induced apoptosis but not cell cycle arrest. Inhibition of endogenous ASPP1 (PPP1R13B) function inhibits the apoptotic function of endogenous p53 in response to apoptotic stimuli. ASPP1 amplifies DNA binding and transactivation function of p53 on the promoters of proapoptotic genes *in vivo*. Expression of ASPP1 is often downregulated in human breast carcinomas expressing wildtype p53, but not in those expressing mutant p53. This research indicates that ASPP1 regulates the tumor suppression function of p53 *in vivo*. ASPP1 is predominantly a cytoplasmic protein, although some fraction of the polypeptide is nuclear. Defects in PPP1R13B, the gene which encodes ASPP1, may be a cause of breast cancers. The deduced ASPP1 protein contains 1,090 amino acid residues.

REFERENCES

1. Nagase, T., et al. 1999. Prediction of the coding sequences of unidentified human genes. XI. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. DNA Res. 5: 277-286.
2. Samuels-Lev, Y., et al. 2001. ASPP proteins specifically stimulate the apoptotic function of p53. Mol. Cell 8: 781-794.
3. Bergamaschi, D., et al. 2004. ASPP1 and ASPP2: common activators of p53 family members. Mol. Cell. Biol. 24: 1341-1350.
4. Bergamaschi, D., et al. 2005. MDM2 and MDMX prevent ASPP1 and ASPP2 from stimulating p53 without targeting p53 for degradation. Oncogene 24: 3836-3841.
5. Fogal, V., et al. 2005. ASPP1 and ASPP2 are new transcriptional targets of E2F. Cell Death Differ. 12: 369-376.
6. Agirre, X., et al. 2006. ASPP1, a common activator of TP53, is inactivated by aberrant methylation of its promoter in acute lymphoblastic leukemia. Oncogene 25: 1862-1870.

CHROMOSOMAL LOCATION

Genetic locus: PPP1R13B (human) mapping to 14q32.33.

PRODUCT

ASPP1 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 ASPP1 shRNA Plasmid (h): sc-60214-SH and ASPP1 shRNA (h) Lentiviral Particles: sc-60214-V as alternate gene silencing products.

For independent verification of ASPP1 (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-60214A, sc-60214B and sc-60214C.

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

ASPP1 siRNA (h) is recommended for the inhibition of ASPP1 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

ASPP1 (LX011): sc-53903 is recommended as a control antibody for monitoring of ASPP1 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 ASPP1 gene expression knockdown using RT-PCR Primer: ASPP1 (h)-PR: sc-60214-PR (20 µl, 431 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.