SANTA CRUZ BIOTECHNOLOGY, INC.

XAF1 siRNA (h): sc-37511

BACKGROUND

X-linked inhibitor of apoptosis protein (XIAP)-associated factor 1 (XAF1) is a zinc-finger protein that blocks the anti-apoptotic activity of XIAP. XIAP is a member of the family of intrinsic inhibitors of apoptosis proteins (IAPs), which suppress apoptosis through the inhibition of caspases. In the presence of XAF1, XIAP protein redistributes from the cytosol to the nucleus. XAF1 transcripts (3.9-, 4.5-, 6.0- and 7.0-kb) are present at high levels in heart and ovary. Low expression of XAF1 mRNA is an indicator for certain cancers (WM164 melanoma, WM35 melanoma, U937 pro-monocytic leukemia and HT1080 fibrosarcoma), suggesting that low levels of XAF1 transcript may enhance cancer cell-survival through the relative increase in XIAP anti-apoptotic function. IFN-α and IFN-β activate the human XAF1 gene, which maps to chromosome 17p13.1.

REFERENCES

1. Fong, W.G., et al. 2000. Expression and genetic analysis of XIAP-associated factor 1 (XAF1) in cancer cell lines. Genomics 70: 113-122.
2. Holcik, M., et al. 2001. XIAP: apoptotic brake and promising therapeutic target. Apoptosis 6: 253-261.
3. Liston, P., et al. 2001. Identification of XAF1 as an antagonist of XIAP anti-caspase activity. Nat. Cell Biol. 3: 128-133.

CHROMOSOMAL LOCATION

Genetic locus: XAF1 (human) mapping to 17p13.1.

PRODUCT

XAF1 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 XAF1 shRNA Plasmid (h): sc-37511-SH and XAF1 shRNA (h) Lentiviral Particles: sc-37511-V as alternate gene silencing products.

For independent verification of XAF1 (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-37511A, sc-37511B and sc-37511C.

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

XAF1 siRNA (h) is recommended for the inhibition of XAF1 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

XAF1 (B-5): sc-398012 is recommended as a control antibody for monitoring of XAF1 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 XAF1 gene expression knockdown using RT-PCR Primer: XAF1 (h)-PR: sc-37511-PR (20 µl, 533 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Hervouet, E., et al. 2010. Impact of the DNA methyltransferases expression on the methylation status of apoptosis-associated genes in glioblastoma multiforme. Cell Death Dis. 1: e8.
2. Zou, B., et al. 2012. XIAP-associated factor 1 (XAF1), a novel target of p53, enhances p53-mediated apoptosis via post-translational modification. Mol. Carcinog. 51: 422-432.
3. Reich, T.R., et al. 2017. Epigenetic silencing of XAF1 in high-grade gliomas is associated with IDH1 status and improved clinical outcome. Oncotarget 8: 15071-15084.
4. Steigerwald, C., et al. 2018. Sensitization of colorectal cancer cells to irinotecan by the survivin inhibitor LLP3 depends on XAF1 proficiency in the context of mutated p53. Arch. Toxicol. 92: 2645-2648.

RESEARCH USE

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