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XAF1 siRNA (m): sc-155371

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 17p13.2.

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.
4. Perrelet, D., et al. 2002. IAPs are essential for GDNF-mediated neuroprotective effects in injured motor neurons *in vivo*. *Nat. Cell Biol.* 4: 175-179.
5. Leaman, D.W., et al. 2002. Identification of X-linked inhibitor of apoptosis-associated factor-1 as an interferon-stimulated gene that augments TRAIL Apo2L-induced apoptosis. *J. Biol. Chem.* 277: 28504-28511.
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CHROMOSOMAL LOCATION

Genetic locus: Xaf1 (mouse) mapping to 11 B4.

PRODUCT

XAF1 siRNA (m) is a pool of 2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see XAF1 shRNA Plasmid (m): sc-155371-SH and XAF1 shRNA (m) Lentiviral Particles: sc-155371-V as alternate gene silencing products.

For independent verification of XAF1 (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-155371A and sc-155371B.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

XAF1 siRNA (m) is recommended for the inhibition of XAF1 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 μ M in 66 μ l. 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor XAF1 gene expression knockdown using RT-PCR Primer: XAF1 (m)-PR: sc-155371-PR (20 μ l). 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.