SANTA CRUZ BIOTECHNOLOGY, INC.

Daxx siRNA (h): sc-35178

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The Power to Question

BACKGROUND

Activation of the cell surface receptor Fas by Fas ligand leads to the initiation of apoptosis, a process necessary for the regulation of the immune system and tissue homeostasis. Fas-mediated apoptosis appears to involve a number of divergent and overlapping pathways. Daxx appears to be a central component of a Fas-mediated apoptotic pathway involving the activation of Jun N-terminal kinase (JNK). Although Daxx itself does not contain a death domain, it specifically binds to the death domain of Fas. Overexpression of Daxx activates the JNK pathway and enhances Fas-mediated apoptosis. The Daxx apoptotic pathway acts cooperatively with but is distinct from the Fas-mediated pathway that involves interactions between the death domain-containing protein FADD and the cysteine protease FLICE. Unlike the Fas-FADD-FLICE pathway, the Daxx pathway is sensitive to the apoptotic inhibitor protein Bcl-2.

REFERENCES

1. Chinnaiyan, A.M., et al. 1995. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. Cell 81: 505-512.
2. Hsu, H., et al. 1996. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. Cell 84: 299-308.
3. Fraser, A. and Evan, G. 1996. A license to kill. Cell 85: 781-784.
4. Boldin, M.P., et al. 1996. Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. Cell 85: 803-815.

CHROMOSOMAL LOCATION

Genetic locus: DAXX (human) mapping to 6p21.32.

PRODUCT

Daxx siRNA (h) is a target-specific 19-25 nt siRNA 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 Daxx shRNA Plasmid (h): sc-35178-SH and Daxx shRNA (h) Lentiviral Particles: sc-35178-V as alternate gene silencing 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

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

Daxx (H-7): sc-8043 is recommended as a control antibody for monitoring of Daxx 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 Daxx gene expression knockdown using RT-PCR Primer: Daxx (h)-PR: sc-35178-PR (20 µl, 478 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Ryo, A., et al. 2007. A suppressive role of the prolyl isomerase Pin1 in cellular apoptosis mediated by the death-associated protein Daxx. J. Biol. Chem. 282: 36671-36681.
2. Sharma, R., et al. 2008. 4-Hydroxynonenal self-limits Fas-mediated DISC-independent apoptosis by promoting export of Daxx from the nucleus to the cytosol and its binding to Fas. Biochemistry 47: 143-156.
3. Jia, L., et al. 2008. Critical roles for JNK, c-Jun, and Fas/FasL-signaling in vitamin E analog-induced apoptosis in human prostate cancer cells. Prostate 68: 427-441.
4. Pan, W.W., et al. 2013. Death domain-associated protein Daxx promotes ovarian cancer development and chemoresistance. J. Biol. Chem. 288: 13620-13630.

RESEARCH USE

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