

▶ XIAP siRNA (m): sc-37509

BACKGROUND

The baculovirus protein p35 inhibits virally induced apoptosis of invertebrate and mammalian cells and may function to impair the clearing of virally infected cells by the immune system of the host. This is accomplished at least in part by its ability to block both TNF- and FAS-mediated apoptosis through the inhibition of the ICE family of serine proteases. Two mammalian homologs of baculovirus p35, referred to as inhibitor of apoptosis protein (IAP) 1 and 2, share an amino-terminal baculovirus IAP repeat (BIR) motif and a carboxy-terminal RING finger. Although the c-IAPs do not directly associate with the TNF receptor (TNF-R), they efficiently block TNF-mediated apoptosis through their interaction with the downstream TNF-R effectors, TRAF1 and TRAF2. Additional IAP family members include XIAP and survivin. XIAP inhibits activated caspase-3, leading to the resistance of FAS-mediated apoptosis. Survivin (also designated TIAP) is expressed during the G₂/M phase of the cell cycle and associates with microtubules of the mitotic spindle. Increased caspase-3 activity is detected when a disruption of survivin-microtubule interactions occurs.

REFERENCES

1. Hay, B.A., et al. 1994. Expression of baculovirus p35 prevents cell death in *Drosophila*. *Development* 120: 2121-2129.
2. Beidler, D.R., et al. 1995. The baculovirus p35 protein inhibits FAS- and tumor necrosis factor-induced apoptosis. *J. Biol. Chem.* 270: 16526-16528.
3. Rothe, M., et al. 1995. The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. *Cell* 83: 1243-1252.
4. Bump, N.J., et al. 1995. Inhibition of ICE family proteases by baculovirus antiapoptotic protein p35. *Science* 269: 1885-1888.
5. Uren, A.G., et al. 1996. Cloning and expression of apoptosis inhibitory protein homologs that function to inhibit apoptosis and/or bind tumor necrosis factor receptor-associated factors. *Proc. Natl. Acad. Sci. USA* 93: 4974-4978.
6. Suzuki, A., et al. 1998. Resistance to FAS-mediated apoptosis: activation of caspase 3 is regulated by cell cycle regulator p21^{WAF1} and IAP gene family ILP. *Oncogene* 17: 931-939.

CHROMOSOMAL LOCATION

Genetic locus: Xiap (mouse) mapping to X A4.

PRODUCT

XIAP 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see XIAP shRNA Plasmid (m): sc-37509-SH and XIAP shRNA (m) Lentiviral Particles: sc-37509-V as alternate gene silencing products.

For independent verification of XIAP (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-37509A, sc-37509B and sc-37509C.

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

XIAP siRNA (m) is recommended for the inhibition of XIAP 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

XIAP (A-7): sc-55550 is recommended as a control antibody for monitoring of XIAP 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 XIAP gene expression knockdown using RT-PCR Primer: XIAP (m)-PR: sc-37509-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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.