SANTA CRUZ BIOTECHNOLOGY, INC.

BAR siRNA (h): sc-37291

BACKGROUND

Two converging apoptotic pathways, which are initiated either through the activation of death domain (DD) receptors by an extrinsic pathway or by an intrinsic pathway in the mitochondria, mediate the activation and progression of apoptosis within the cell. Both these pathways lead to the activation of the Serine proteinase cascade (caspases) and to cleavage of these pro-caspases. A novel protein, BAR, for bifunctional apoptosis regulator, contains domains that enable it to interact with components of both major apoptosis pathways, where it negatively regulates apoptotic signaling. Like the other anti-apoptosis proteins Bap31 and FLIP, BAR contains a DED-like domain that is capable of suppressing apoptosis mediated at the receptor level. In addition, BAR contains a domain that also enables it to interact with the mitochondrial Bcl-2 family of proteins. The presence of these various RING, SAM, DED and TM domains suggests that BAR may serve as a scaffold protein that integrates signaling components of the cells apoptosis-regulatory machinery.

REFERENCES

1. Irmler, M., et al. 1997. Inhibition of death receptor signals by cellular FLIP. Nature 388: 190-195.
2. Chao, D.T., et al. 1998. Bcl-2 family: regulators of cell death. Annu. Rev. Immunol. 16: 395-419.
3. Ng, F.W., et al. 1998. Bcl-x_L cooperatively associates with the Bap31 complex in the endoplasmic reticulum, dependent on procaspase-8 and Ced-4 adaptor. J. Biol. Chem. 273: 3140-3143.
4. Zhang, H., et al. 2000. BAR: An apoptosis regulator at the intersection of caspases and Bcl-2 family proteins. Proc. Natl. Acad. Sci. USA 97: 2597-2602.
5. Reed, J.C. 2000. Mechanisms of apoptosis. Am. J. Pathol. 157: 1415-1430.
6. Roy, S., et al. 2000. Programmed cell-death regulation: basic mechanisms and therapeutic opportunities. Mol. Med. Today 6: 264-266.
7. Stegh, A.H., et al. 2002. Inactivation of caspase-8 on mitochondria of Bcl-x_L-expressing MCF7-Fas cells: role for the bifunctional apoptosis regulator protein. J. Biol. Chem. 277: 4351-4360.
8. Roth, W., et al. 2003. Bifunctional apoptosis inhibitor (BAR) protects neurons from diverse cell death pathways. Cell Death Differ. 10: 1178-1187.

CHROMOSOMAL LOCATION

Genetic locus: BFAR (human) mapping to 16p13.12.

PRODUCT

BAR 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 BAR shRNA Plasmid (h): sc-37291-SH and BAR shRNA (h) Lentiviral Particles: sc-37291-V as alternate gene silencing products.

For independent verification of BAR (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-37291A, sc-37291B and sc-37291C.

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

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

BAR (L-16): sc-101217 is recommended as a control antibody for monitoring of BAR 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 BAR gene expression knockdown using RT-PCR Primer: BAR (h)-PR: sc-37291-PR (20 µl, 557 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.

PROTOCOLS

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