

BAP31 siRNA (m): sc-37284

BACKGROUND

BAP31, a human Bcl-2-interacting protein, is an integral membrane protein that is a component of a protein complex in the endoplasmic reticulum. This protein complex mechanically bridges an apoptosis-initiating caspase, like procaspase-8, with the anti-apoptotic regulator Bcl-2 or Bcl-x_L. The cytosolic domain of BAP31 contains two identical caspase recognition sites, which are preferentially cleaved by initiator caspases, including caspase 8. Cleavage of BAP31 during apoptosis generates a p20 fragment, which remains integrated in the membrane and, when expressed ectopically, is a potent inducer of cell death. BAP31 cleavage is important for manifesting cytoplasmic apoptotic events associated with membrane fragmentation and in the cross talk between mitochondria and the endoplasmic reticulum during Fas-mediated apoptosis. The BAP31 gene is ubiquitously expressed in murine tissues and is located on the X chromosome in both mouse and human.

REFERENCES

1. Adachi, T., et al. 1996. The specificity of association of the IgD molecule with the accessory proteins BAP31/BAP29 lies in the IgD transmembrane sequence. *EMBO J.* 15: 1534-1541.
2. Ng, F.W., et al. 1997. p28 BAP31, a Bcl-2/Bcl-x_L- and procaspase-8-associated protein in the endoplasmic reticulum. *J. Cell Biol.* 139: 327-338.
3. Annaert, W.G., et al. 1997. Export of cellubrevin from the endoplasmic reticulum is controlled by BAP31. *J. Cell Biol.* 139: 1397-1410.
4. Granville, D.J., et al. 1998. Rapid cytochrome c release, activation of caspases 3, 6, 7 and 8 followed by BAP31 cleavage in HeLa cells treated with photodynamic therapy. *FEBS Lett.* 437: 5-10.
5. Nguyen, M., et al. 2000. Caspase-resistant BAP31 inhibits Fas-mediated apoptotic membrane fragmentation and release of cytochrome c from mitochondria. *Mol. Cell. Biol.* 20: 6731-6740.

CHROMOSOMAL LOCATION

Genetic locus: Bcap31 (mouse) mapping to X A7.3.

PRODUCT

BAP31 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 BAP31 shRNA Plasmid (m): sc-37284-SH and BAP31 shRNA (m) Lentiviral Particles: sc-37284-V as alternate gene silencing products.

For independent verification of BAP31 (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-37284A, sc-37284B and sc-37284C.

PROTOCOLS

See our web site at www.scbt.com 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

BAP31 siRNA (m) is recommended for the inhibition of BAP31 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

BAP31 (B-10): sc-365347 is recommended as a control antibody for monitoring of BAP31 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 BAP31 gene expression knockdown using RT-PCR Primer: BAP31 (m)-PR: sc-37284-PR (20 μ l, 568 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.