

caspase-3 siRNA (m): sc-29927

BACKGROUND

Caspase-3, also known as apopain, SCA-1, Yama and CPP32, is an aspartate-specific cysteine protease that belongs to the ICE subfamily of caspases. Caspase-3 is expressed in cells as an inactive precursor from which the p17 and p11 subunits of the mature caspase-3 are proteolytically generated during apoptosis. The caspase-3 precursor is first cleaved at Asp 175-Ser 176 to produce the p11 subunit and the p20 peptide. Subsequently, the p20 peptide is cleaved at Asp 28-Ser 29 to generate the mature p17 subunit. The active caspase-3 enzyme is a heterodimer composed of two p17 and two p11 subunits. At the onset of apoptosis, caspase-3 proteolytically cleaves PARP at a Asp 216-Gly 217 bond. During the execution of the apoptotic cascade, activated caspase-3 releases SREBP from the membrane of the ER in a proteolytic reaction that is distinct from their normal sterol-dependent activation. Caspase-3 cleaves and activates SREBPs between the basic helix-loop-helix leucine zipper domain and the membrane attachment domain. Caspase-3 also cleaves and activates caspase-6, -7 and -9. The human caspase-3 gene encodes a cytoplasmic protein that is highly expressed in lung, spleen, heart, liver, kidney and cells of the immune system.

CHROMOSOMAL LOCATION

Genetic locus: Casp3 (mouse) mapping to 8 B1.1.

PRODUCT

caspase-3 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 caspase-3 shRNA Plasmid (m): sc-29927-SH and caspase-3 shRNA (m) Lentiviral Particles: sc-29927-V as alternate gene silencing products.

For independent verification of caspase-3 (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-29927A, sc-29927B and sc-29927C.

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

caspase-3 siRNA (m) is recommended for the inhibition of caspase-3 expression in mouse cells.

PROTOCOLS

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

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

caspase-3 p11 (C-6): sc-271759 is recommended as a control antibody for monitoring of caspase-3 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 λ BP-HRP: sc-516132 or m-IgG λ BP-HRP (Cruz Marker): sc-516132-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 λ BP-FITC: sc-516185 or m-IgG λ BP-PE: sc-516186 (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 caspase-3 gene expression knockdown using RT-PCR Primer: caspase-3 (m)-PR: sc-29927-PR (20 μ l, 491 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Kim, K.W., et al. 2008. M867, a novel selective inhibitor of caspase-3 enhances cell death and extends tumor growth delay in irradiated lung cancer models. *PLoS ONE* 3: e2275.
- Chu, J., et al. 2015. γ secretase activating protein is a substrate for caspase-3: implications for Alzheimer's disease. *Biol. Psychiatry* 77: 720-728.
- Chu, J., et al. 2015. Regulation of γ -secretase activating protein by the 5Lipoxygenase: *in vitro* and *in vivo* evidence. *Sci. Rep.* 5: 11086.
- Chu, J., et al. 2017. Caspase-3-dependent cleavage of Akt modulates tau phosphorylation via GSK3 β kinase: implications for Alzheimer's disease. *Mol. Psychiatry* 22: 1002-1008.
- Wang, C., et al. 2020. Inhibition of caspases improves non-viral T cell receptor editing. *Cancers* 12: E2603.

RESEARCH USE

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