

caspase-2 siRNA (r): sc-72108

BACKGROUND

Caspase-2 (Nedd2, ICH-1) is an aspartate-specific cysteine protease that is activated in response to various apoptotic stimuli. Caspase-2 is unique among the caspases in that it has features of both upstream caspases (long prodomain) and downstream caspases (DEXD substrate specificity). Caspase-2 is highly expressed in the brain during development and is expressed at low levels in adult tissue. Specifically, caspase-2 localizes to the mitochondria, the Golgi, the cytoplasm and the nucleus. Caspase-2 exists as two isoforms, caspase-2L and caspase-2S, which are produced by alternative splicing and differ in their N- and C-termini. Caspase-2L acts as a positive regulator of apoptosis, whereas caspase-2S functions as a negative regulator of apoptosis. Following apoptotic stimuli, the caspase-2L precursor undergoes cleavage at Asp-153 to produce a fragment (p30). The p30 fragment undergoes further cleavage to generate a fragment containing amino acids 153-308 (p18) and a fragment containing amino acids 317-435 (p13 or p14). As apoptosis progresses, the p13 (p14) fragment can undergo further processing to yield a fragment containing amino acids 331-435 (p12).

REFERENCES

1. Wang, L., et al. 1994. Ich-1, an Ice/ced-3-related gene, encodes both positive and negative regulators of programmed cell death. *Cell* 78: 739-750.
2. Li, H., et al. 1997. Activation of caspase-2 in apoptosis. *J. Biol. Chem.* 272: 21010-21017.
3. Butt, A., et al. 1998. Dimerization and autoprocessing of the Nedd2 (caspase-2) precursor requires both the prodomain and the carboxyl-terminal regions. *J. Biol. Chem.* 273: 6763-6768.

CHROMOSOMAL LOCATION

Genetic locus: Casp2 (rat) mapping to 4q23.

PRODUCT

caspase-2 siRNA (r) 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-2 shRNA Plasmid (r): sc-72108-SH and caspase-2 shRNA (r) Lentiviral Particles: sc-72108-V as alternate gene silencing products.

For independent verification of caspase-2 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-72108A, sc-72108B and sc-72108C.

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-2 siRNA (r) is recommended for the inhibition of caspase-2 expression in rat 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

caspase-2L (F-7): sc-5292 is recommended as a control antibody for monitoring of caspase-2 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 caspase-2 gene expression knockdown using RT-PCR Primer: caspase-2 (r)-PR: sc-72108-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Vigneswara, V. and Ahmed, Z. 2019. Pigment epithelium-derived factor mediates retinal ganglion cell neuroprotection by suppression of caspase-2. *Cell Death Dis.* 10: 102.

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.