

caspase-4/5 p10 siRNA (h): sc-43668

BACKGROUND

Caspases are cysteine proteases which play important roles in the activation of cytokines and in apoptosis. The ICE subfamily of caspases comprises peptides closely related to caspase-1, which promotes maturation of interleukin 1 β (IL-1 β) and interleukin-18 (IL-18) by proteolytic cleavage of precursor forms to generate biologically active peptides. Both caspase-4 and caspase-5 are members of the caspase-1 subfamily, and are more closely related to each other than to other homologs. Caspase-5 (also designated ICErel-III, TY, ICH-3 and caspase-12 in mouse), can cleave its own precursor, an activity that requires the Cysteine 245 residue. The human caspase-5 gene maps to chromosome 11q22.3 and encodes a protein whose expression is barely detectable in most tissues except brain. Caspase-4 cleaves caspase-1 and has two subunits derived from the precursor sequence by an autocatalytic mechanism or by cleavage by caspase-8. Caspase-4 is widely expressed, with highest levels in spleen and lung, and is not found in the brain.

REFERENCES

1. Lindahl, T., et al. 1995. Post-translational modification of poly (ADP-ribose) polymerase induced by DNA strand breaks. *Trends Biochem. Sci.* 20: 405-411.
2. Duan, H., et al. 1996. ICE-LAP3, a novel mammalian homologue of the *Caenorhabditis elegans* cell death protein Ced-3 is activated during Fas- and tumor necrosis factor-induced apoptosis. *J. Biol. Chem.* 271: 1621-1625.
3. Fernandes-Alnemri, T.F., et al. 1996. *In vitro* activation of CPP32 and Mch3 by Mch4, a novel human apoptotic cysteine protease containing two FADD-like domains. *Proc. Natl. Acad. Sci. USA* 93: 7464-7469.
4. Duan, H., et al. 1996. ICE-LAP6, a novel member of the ICE/Ced-3 gene family, is activated by the cytotoxic T cell protease granzyme B. *J. Biol. Chem.* 271: 16720-16724.
5. Simbulan-Rosenthal, C.M., et al. 1996. The expression of poly(ADP-ribose) polymerase during differentiation-linked DNA replication complex. *Biochem.* 35: 11622-11633.
6. Casciola-Rosen, L., et al. 1996. Apopain/CPP32 cleaves proteins that are essential for cellular repair: a fundamental principle of apoptotic death. *J. Exp. Med.* 183: 1957-1964.

CHROMOSOMAL LOCATION

Genetic locus: CASP5 (human) mapping to 11q22.3.

PRODUCT

caspase-4/5 p10 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see caspase-4/5 p10 shRNA Plasmid (h): sc-43668-SH and caspase-4/5 p10 shRNA (h) Lentiviral Particles: sc-43668-V as alternate gene silencing products. For independent verification of caspase-4/5 p10 (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-43668A, sc-43668B and sc-43668C.

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-4/5 p10 siRNA (h) is recommended for the inhibition of caspase-4/5 p10 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 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-5 p20 (H-2): sc-393346 is recommended as a control antibody for monitoring of caspase-4/5 p10 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-4/5 p10 gene expression knockdown using RT-PCR Primer: caspase-4/5 p10 (h)-PR: sc-43668-PR (20 μ l, 433 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.