SANTA CRUZ BIOTECHNOLOGY, INC.

CAD siRNA (m): sc-29872

BACKGROUND

The Ced/ICE or caspase family of cysteine proteases plays a pivotol role in mediating apoptosis through the proteolysis of specific targets. Among the targets are poly(ADP-ribose) polymerase (PARP), Gelsolin, ICAD (inhibitor of CAD, also designated DFF-45) and the nuclear lamins. CAD (caspase-activated deoxyribonuclease), also designated CPAN (caspase-activated nuclease) and DFF40, is a DNase that is responsible for DNA degradation during apoptosis. Caspase-3 acts to dissociate CAD from ICAD, allowing CAD to enter the nucleus and degrade DNA.

REFERENCES

1. Fernandes-Alnemri, T., et al. 1995. MCH3, a novel human apoptotic cysteine protease highly related to CPP32. Cancer Res. 55: 6045-6052.
2. Takahashi, A., et al. 1996. Cleavage of lamin A by Mch2 α but not CPP32: multiple interleukin 1 β-converting enzyme-related proteases with distinct substrate recognition properties are active in apoptosis. Proc. Natl. Acad. Sci. USA 93: 8395-8400.
3. Liu, X., et al. 1997. DFF, a hetrodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. Cell 89: 175-184.
4. Salvesen, G.S., et al. 1997. Caspases: intracellular signaling by proteolysis. Cell 91: 443-446.
5. Kothakota, S., et al. 1997. Caspase-3-generated fragment of gelsolin: effector of morphological change in apoptosis. Science 278: 294-298.
6. Enari, M., et al. 1998. A caspase-activated Dnase that degrades DNA during apoptosis. Nature 391: 43-50.
7. Sakahira, H., et al. 1998. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature 391: 96-99.
8. Halenbeck, R., et al. 1998. CPAN, a human nuclease regulated by the caspase-sensitive inhibitor DFF45. Curr. Biol. 8: 537-540.
9. Shiokawa, D., et al. 2007. Stage-specific expression of DNaseγ during B-cell development and its role in B-cell receptor-mediated apoptosis in WEHI-231 cells. Cell Death Differ. 14: 992-1000.

CHROMOSOMAL LOCATION

Genetic locus: Dffb (mouse) mapping to 4 E2.

PRODUCT

CAD 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CAD shRNA Plasmid (m): sc-29872-SH and CAD shRNA (m) Lentiviral Particles: sc-29872-V as alternate gene silencing products.

For independent verification of CAD (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-29872A, sc-29872B and sc-29872C.

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

CAD shRNA (m) Lentiviral Particles is recommended for the inhibition of CAD 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 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

CAD (F-11): sc-374067 is recommended as a control antibody for monitoring of CAD 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 CAD gene expression knockdown using RT-PCR Primer: CAD (m)-PR: sc-29872-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Lovric, M.M. and Hawkins, C.J. 2010. TRAIL treatment provokes mutations in surviving cells. Oncogene 29: 5048-5060.

RESEARCH USE

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