SANTA CRUZ BIOTECHNOLOGY, INC.

ICAD siRNA (m): sc-35625

BACKGROUND

The CED/ICE family of cysteine proteases plays a pivotal role in mediating apoptosis through the proteolysis of specific targets. Among the targets are poly (ADP-ribose) polymerase (PARP), gelsolin, DFF-45/ICAD and the nuclear lamins. PARP is a nuclear protein that is specifically cleaved by CPP32 and Mch2, but not by ICE, into a signature apoptotic fragment. Gelsolin is cleaved by CPP32 to an active form that severs actin filaments in a Ca^{2+}-independent manner. In addition to binding actin, gelsolin can form complexes with fibronectin, which may be important for localizing gelsolin to inflammatory sites. DFF-45/ICAD, the subunit of DNA fragmentation factor, is cleaved by CPP32 to generate an active factor that induces DNA fragmentation. The nuclear Lamin A is cleaved by Mch2, but not CPP32. Nuclear Lamin B is fragmented as a consequence of apoptosis by an unidentified member of the ICE family .

REFERENCES

1. Lind, S.E., et al. 1984. Human plasma gelsolin binds to fibronectin. J. Biol. Chem. 259 13262-13266.
2. Takahashi, A., et al. 1996. Cleavage of Lamin A by Mch2 α but not CPP32: multiple interleukin 1β-converting enzyme-related proteases with distinct substrate recognition properties are active in apoptosis. Proc. Natl. Acad. Sci. USA 93: 8395-8400.
3. Salvesen, G.S., et al. 1997. Caspases: intracellular signaling by proteolysis. Cell 91: 443-446.
4. Eckhart, L., et al. 2007. Phylogenomics of caspase-activated DNA fragmentation factor. Biochem. Biophys. Res. Commun. 356: 293-299.
5. Karmakar, S., et al. 2007. Garlic compounds induced calpain and intrinsic caspase cascade for apoptosis in human malignant neuroblastoma SH-SY5Y cells. Apoptosis 12: 671-684.
6. Shiokawa, D., et al. 2007. Stage-specific expression of DNasegamma during B-cell development and its role in B-cell receptor-mediated apoptosis in WEHI-231 cells. Cell Death Differ. 14: 992-1000.
7. Cullen, S.P., et al. 2007. Human and murine granzyme B exhibit divergent substrate preferences. J. Cell Biol. 176:435-444.

CHROMOSOMAL LOCATION

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

PRODUCT

ICAD 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 ICAD shRNA Plasmid (m): sc-35625-SH and ICAD shRNA (m) Lentiviral Particles: sc-35625-V as alternate gene silencing products.

For independent verification of ICAD (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-35625A, sc-35625B and sc-35625C.

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

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

$ICAD_L$ (C-4): sc-376044 is recommended as a control antibody for monitoring of ICAD 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 ICAD gene expression knockdown using RT-PCR Primer: ICAD (m)-PR: sc-35625-PR (20 µl). 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.