

ICAD (D-6): sc-398431

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. Fernandes-Alnemri, T., et al. 1995. Mch3, a novel human apoptotic cysteine protease highly related to CPP32. *Cancer Res.* 55: 6045-6052.
3. 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.
4. Rao, L., et al. 1996. Lamin proteolysis facilitates nuclear events during apoptosis. *J. Cell Biol.* 135: 1441-1455.
5. Liu, X., et al. 1997. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell* 89: 175-184.
6. Kothakota, S., et al. 1997. Caspase-3-generated fragment of gelsolin: effector of morphological change in apoptosis. *Science* 278: 294-298.
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 (human) mapping to 1p36.22; Dffa (mouse) mapping to 4 E2.

SOURCE

ICAD (D-6) is a mouse monoclonal antibody specific for an epitope mapping between amino acids 199-226 near the C-terminus of ICAD of human origin.

PRODUCT

Each vial contains 200 μ g IgG $_1$ kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-398431 P, (100 μ g peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% stabilizer protein).

APPLICATIONS

ICAD (D-6) is recommended for detection of ICAD and DFF-35 splice variant of mouse, rat and human origin by Western Blotting (starting dilution 1:100, dilution range 1:100-1:1000), immunoprecipitation [1-2 μ g per 100-500 μ g of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

Suitable for use as control antibody for ICAD siRNA (h): sc-35624, ICAD siRNA (m): sc-35625, ICAD shRNA Plasmid (h): sc-35624-SH, ICAD shRNA Plasmid (m): sc-35625-SH, ICAD shRNA (h) Lentiviral Particles: sc-35624-V and ICAD shRNA (m) Lentiviral Particles: sc-35625-V.

Molecular Weight of DFF-45 splice variant: 45 kDa.

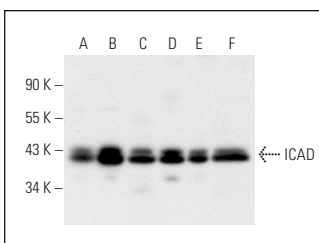
Molecular Weight of DFF-35 splice variant: 35 kDa.

Positive Controls: K-562 whole cell lysate: sc-2203, Jurkat whole cell lysate: sc-2204 or HT-1080 whole cell lysate: sc-364183.

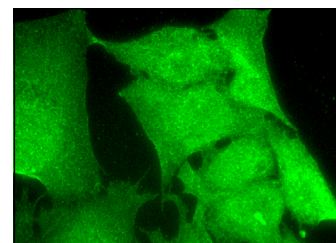
RECOMMENDED SUPPORT REAGENTS

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) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) 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.

DATA



ICAD (D-6): sc-398431. Western blot analysis of ICAD expression in Jurkat (A), K-562 (B), U-937 (C), HT-1080 (D), A-431 (E) and HeLa (F) whole cell lysates.



ICAD (D-6): sc-398431. Immunofluorescence staining of formalin-fixed Hep G2 cells showing cytoplasmic and nuclear localization.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

RESEARCH USE

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