ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Life Technologies™ Your Molecular & Cell Technology Partner

Caspase-9/p35 (CASP-9/MHC-6) Antibodies

Cat. # CASP92-P	Human Casp-9/p35 Control Peptide # 2	**SIZE**: 100 ug
Cat. # CASP92-S	Rabbit Anti-Human Casp-9/p35 antiserum # 2	**SIZE**: 100 ul
Cat. # CASP92-A	Rabbit Anti-Human Casp-9/p35 IgG # 2 (aff pure)	**SIZE**: 100 ug

Apoptosis or programmed cell death is a fundamental cellular process that is essential for normal tissue development and abnormal growth such as cancer, neurodegeneration, autoimmune diseases, and angiogenesis, etc. Apoptosis is driven by specialized proteases known as **caspases**. After the initial discovery of the first mammalian caspase 1 or ICE (interleukin 1 beta converting enzyme), a growing family of **caspases 1-14** have been cloned and characterized. Caspases are synthesized as inactive zymogen or proenzyme forms (30-55 kDa), which upon apoptotic stimulation are proteolytically processed (self or by other proteins) in a sequential manner into their active heterotetrameric forms. The processed form consists of large subunit (17-20 kDa) and a small (10-12 kDa) subunits, which may associate to form an active enzyme. Functionally active caspases initiate a proteolytic cascade, capable of cleaving and activating numerous cellular targets including PARP, G4-GDI, DFF, MEKK, etc. On a functional basis, two categories of caspases have been defined: the **initiator caspases** (caspases-8, -9, and –10) are activated in the earlier phases of apoptosis, whereas the **executioner caspases** (caspases-3, -6, and –7) are activated by initiator caspases and are responsible for dismantling cellular components. Caspases are widely distributed in various tissues and cells.

Caspase-9 (rat/mouse 454 aa; human 416-aa, also known as **apoptotic protease activating factor 3 (APAF-3)/ICE-like apoptotic protease 6 or ICE-LAP6 or MCH-6**, is involved in the activation cascade of caspases responsible for apoptosis execution. Binding of Caspase-9 to Apaf-1 leads to activation of the protease which then cleaves and activates caspase-3. Casp-9 proteolytically cleaves poly ADP-ribose polymerase (PARP). It is a heterodimer of a ~35 kDa (p35) and 10 kDa (p10) subunit. Proteolytic cleavages at Asp315 by granenzyme B and at Asp330 by CPP32 generate the two active subunits. Caspase-8 and –10 may also be involved in these processing events. Caspase-9 is ubiquitous with highest expression in the heart, and moderate expression in liver and skeletal muscle.

Source of Antigen and Antibodies

Antigen	13-aa peptide of **human Caspase-9** (1); **Designation (CASP92-P, control peptide**) Epitope location ~ 200-aa region of p35 subunit
Ab Host/type	Rabbit, Polyclonal Unpurified antiserum (cat # CASP92-S) and aff pure IgG (cat # CASP92-A)purified over the antigen column
2AB	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available
-ve control	# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
100ul solution lyophilized powder
Supplied in Buffer: 0.05% azide
Reconstitute powder in 100 ul PBS

Affinity pure IgG
100 ug/100ul solution lyophilized powder
Supplied in **Buffer**: PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide
100 ug/100 ul solution lyophilized powder
Supplied in Buffer: PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

Storage

Short-term: unopened, undiluted liquid vials at 20°C and powder at 4°C or -20°C..

Long-term: at –20°C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20°C or below.
Shipping: 4°C for solutions and room temp for powder

Recommended Usage

Western Blotting (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure antibody using ECL technique). Caspase –9 p35 subunit is ~35 Kda.

ELISA: Control peptide can be used to coat ELISA plates at 1 ug/ml and detected with antibodies (1:10-50K for neat serum and 0.5-1 ug/ml for affinity pure).

Histochemistry & Immunofluorescence: Not tested.

Specificity & Cross-reactivity

Human CASP92-P control peptide is not well conserved in Casp-9 from other species. No significant sequence homology is detected with other caspases. Since CASP92-P Is located at p35 subunit, the antibodies will not detect the p10 subunit. Antibody cross-reactivity in various species has not been studied. Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (detailed protocol is available at the web site).

General References: (1) Duan H et al (196) JBC 271, 16720; Srinivasula SM et al (1996) JBC 271, 27099; Angelastro JM et al (2001) JBC 276, 12190

*This product is for In vitro research use only.

Related material available from ADI
Antibodies AIF, Apaf-1, Cytochrome-C, Caspases, IAPs, Survivin, EPR-1, CARD, Flash, Aven, Livin, Iceberg

CASP92-S-A-P 71214S

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