ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Flice Associated Huge Protein (FLASH) Antibodies

Cat. # FLASH11-P	Mouse FLASH Control blocking/Peptide # 1	**SIZE**: 100 ug
Cat. # FLASH11-S	Rabbit Anti-Mouse FLASH antiserum #1	**SIZE**: 100 ul
Cat. # FLASH11-A	Rabbit Anti-Mouse FLASH IgG # 1 (affinity pure)	**SIZE**: 100 ug

Apoptosis or programmed cell death is a fundamental cellular process that is essential for normal tissue development and abnormal growth. Apoptosis is driven by two classes of specialized proteases known as caspases (**C**ysteine **As**part**ase**). Several key factors are released from the mitochondria that regulate apoptosis. The first such factor (Cytochrome-C) to be described binds to a cytoplasmic scaffolding protein called **Apaf-1** (Apoptosis Protease activating factor-1), **a homolog of C. elegans Ced-4**. Both Apaf-1 and Ced-4 are composed of an N-terminal Caspase Recruitment domain (CARD) linked to a Nucleotide-binding domain (NBD), also known as NB-ARC or NOD domain. Ced-4 and Apaf-1 self-associate via the NBD and activate Casp-3 and –9. In response to certain apoptotic stimuli, Cytochrome-C is released from the mitochondria and binds to Apaf-1 to form a ternary complex with, and activate, the initiator pro-caspase-9. Active caspase-9 then turns on downstream effector caspases, initiating apoptosis. Recently, Additional members of Apaf-1 family, **NOD1**/**CARD4** and **NOD2** have been cloned and characterized.

FLASH or Caspase 8-associted protein 2/**CASP8AP2** (mouse 1962-aa, ~ 220 kDa; human 1982-aa, chromosome 6) contains motif structurally related to CED4/Apaf1 and 2 tandem-repeated DED homologous domains. In adult mouse, highest expression was found in heart, brain, thymus, lung, testis, and spleen, and much lower expression in liver, kidney, and skeletal muscle. Flash binds caspase-8 and Fadd. It also specifically coimmunoprecipitated with activated Fas, suggesting that Casp8ap2 is part of the death-inducing signaling complex (DISC). Inhibition of FLASH expression abolishes TNF-induced NFKB activation in embryonic kidney cells. Expression or overexpression of FLASH activates NFKB through a central oligomerization domain, called the NFKB activation domain (NAD), in a TRAF2-NIK-IKKA-dependent pathway. FLASH coordinates downstream NFKB activity via a TRAF2-dependent pathway in TNF signaling.

Source of Antigen and Antibodies

Antigen	16-aa peptide from **Mouse FLASH** (1); **Designation (FLASH11-P, control peptide**), Epitope location ~ C-terminus
Ab Host/type	Rabbit, Polyclonal Unpurified antiserum (cat # FLASH11-S) and aff pure IgG (cat # FLASH11-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 Antiserum at 1:1K-1:3K; Aff pure at 1-5 ug/ml using ECL technique).

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

Mouse FLASH11-P control peptide sequence is unique to mouse FLASH. 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) Imai Y et al (1999) Nature 398, 777-785; Choi Y-H et al (2001) JBC 276, 25073-25077

*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, Aven, Livin, Iceberg, IPAF

Recycle Immuno blots in Just 5-10 min. (use the same blot for various proteins). (no boiling or pungent mercaptoethanol).

FLASH11-S-A-P 71215S

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