

Product Specification Sheet

**Apaf-1 (Apoptosis Protease Activating Factor-1) Antibodies**

Cat. # APAF11-P	Human Apaf-1 Control blocking/Peptide	<b>SIZE:</b> 100 ug
Cat. # APAF11-A	Rabbit Anti-Human Apaf-1 IgG (aff pure)	<b>SIZE:</b> 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 (Cysteine **Aspartase**). The **initiator caspases** can be activated by self-cleavage. The **effector caspases** are then activated in an amplification cascade. 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. The C-terminal region of Apaf-1 lacks homology with Ced-4 and contains 12 WD-40 repeats. 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. The presence of CARD motifs in a variety of other effector and signaling molecules, including numerous caspases, NF- $\kappa$ B-activating kinase, suggests that other CED-4/Apaf-1 family members likely exist in humans to coordinate downstream stress responses. Recently, Additional members of Apaf-1 family, **NOD1/CARD4** and **NOD2** have been cloned and characterized.

Apaf-1 (human 1205 aa; chromosome 12q23; mouse 1238 aa; rat 1249 aa; mol wt ~130 kDa) has significant (~20%) homology with ced-3 and ced-4. It is a cytoplasmic protein found in most tissues with highest expression in adult spleen and peripheral blood leukocytes, fetal brain, kidney, and lung correlating with high level of apoptosis in these tissues. Apaf-1 participates in the cytochrome-c dependent activation of caspase-3. The N-terminal 85-aa of Apaf-1 show 21% identity with C. elegans CED3. The next 320-aa show ~22% identity with CED4. C-terminus of Apaf-1 comprised of 12 WD repeats (TRP-Asp domains), which mediate protein-protein interactions. Apaf-1 is alternatively spliced to three forms that vary in length.

**Source of Antigen and Antibodies**

<b>Antigen</b>	17-aa peptide from <b>human APAF11 (1); Designation (APAF11-P, control peptide)</b> . Epitope location ~ N-terminus
<b>Ab Host/type</b>	Rabbit, Polyclonal, Aff pure IgG (cat # APAF11-A)
<b>2ab</b>	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available)
<b>-ve control</b>	# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as -ve control

**Form & Storage of Antibodies/Peptide Control**

**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** ~1.0 ug/ml for affinity pure antibody using ECL technique). Apaf-1 is ~130 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 APAF11-P is 100% conserved in mouse and 94% in rat Apaf-1. It has no significant homology with ced-3/4, Nod-1 and NOD-2 family of proteins. 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 (see detailed protocol at:the web site).

**General References:** (1) Cecconi, F. et al (1998) Cell 94, 727-737; Zou et al (1997) Cell 90, 405-413; Soengas MS et al (2001) Nature 409, 207-211; Srinivasulam SM et al (1998) Mol. Cell 1, 949-957; Inohara N et al (1999) JBC 274, 14560-14567; Bertin J et al (1999) JBC 274, 12955-12953; Ogura Y et al (2001) JBC 276, 4812-4818;

\*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, other Apoptosis related proteins

Pre-made **Kidney and BrainBlot** (study distribution of proteins in 12-distinct regions of rat/mouse brain/kidney)

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

APAF11-A-P

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**India Contact:**

**Life Technologies (India) Pvt. Ltd.**

306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi - 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400, Fax: +91-11-42208444  
Email: [customerservice@lifetechindia.com](mailto:customerservice@lifetechindia.com) Website: [www.lifetechindia.com](http://www.lifetechindia.com)