Technical Data Sheet

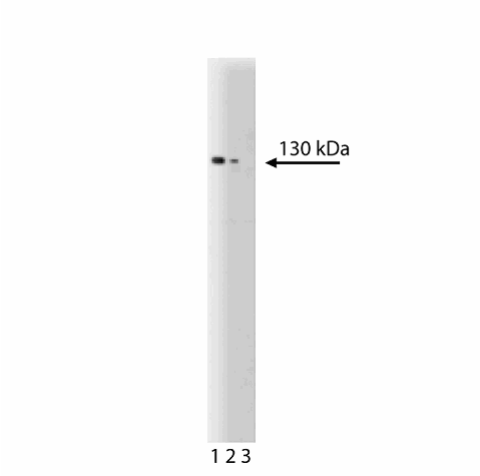
Purified Mouse Anti-Human Apaf-1

Product Information

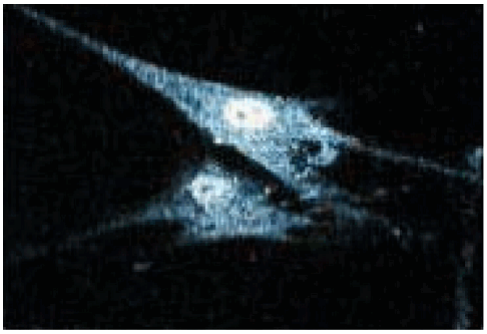
Material Number:	**611365**
Alternate Name:	Apoptotic Protease Activating Factor-1
Size:	150 µg
Concentration:	250 µg/ml
Clone:	24/Apaf-1
Immunogen:	Human Apaf-1 aa. 252-445
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Target MW:	130 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

The process of apoptosis requires the activation of aspartate-specific cystenine proteases in the caspase family. Group I caspases (1,4,5) cleave at (W/L)EHD tetrapeptide motifs, while group II caspases (2,3,7) cleave the DEXD tetrapeptide motif. Group III caspases (6,8,9) are activators of other caspases via cleavage of (I/V)EXD tetrapeptide sequences. Apoptotic protease-activating factor-1 (Apaf-1), cytochrome c, and dATP activate caspase-9, which in turn, initiates the post-mitochondrial-mediated caspase cascade that includes caspase-2, 3, 6, 7, 8 and 10. Apaf-1 is a soluble protein with a short N-terminal caspase recruitment domain (CARD), a central CED-4 homology domain, and 12 WD-40 repeats that may be involved in protein-protein interactions. During apoptosis, a large (700 kDa) aposome complex containing Apaf-1, cytochrome c, caspase-3, 7, and 9, and a smaller (200-300 kDa) microaposome complex containing caspase-3 and 7 exhibit higher cleavage activity than "free" caspase heterotetramers. Thus Apaf-1 is a component of the large aposome complex, which functions in caspase activation leading to caspase-dependent proteolytic events and apoptosis.



Western blot analysis of Apaf-1 on a human endothelial cell lysate. *Lane 1: 1:250, lane 2: 1:500, lane 3: 1:1000 dilution of the mouse anti- Apaf-1 antibody.*



Immunofluorescence staining of WI-38 cells (Human lung fibroblasts; ATCC CCL-75).

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at -20° C.

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Application Notes

Application

Western blot	Routinely Tested
Immunofluorescence	Tested During Development

Recommended Assay Procedure:

Western blot: Please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
611450	Human Endothelial Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Cain K, Brown DG, Langlais C, Cohen GM. Caspase activation involves the formation of the aposome, a large (approximately 700 kDa) caspase-activating complex. *J Biol Chem.* 1999; 274(32):22686-22692.(Biology)

Hu Y, Benedict MA, Ding L, Nunez G. Role of cytochrome c and dATP/ATP hydrolysis in Apaf-1-mediated caspase-9 activation and apoptosis. *EMBO J.* 1999; 18(13):3586-3595.(Biology)

Li CQ, Trudel LJ, Wogan GN. Nitric oxide-induced genotoxicity, mitochondrial damage, and apoptosis in human lymphoblastoid cells expressing wild-type and mutant p53. *Proc Natl Acad Sci U S A.* 2002; 99(16):10364-10369.(Biology: Western blot)

Slee EA, Harte MT, Kluck RM, et al. Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. *J Cell Biol.* 1999; 144(2):281-292.(Biology)

Soengas MS, Alarcon RM, Yoshida H, et al. Apaf-1 and caspase-9 in p53-dependent apoptosis and tumor inhibition. *Science.* 1999; 284(5411):156-159.(Biology)