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Product datasheet for AM26621AF-N

APAF1 (1-421) Mouse Monoclonal Antibody [Clone ID: 5C1]

Product data:

Product Type:	Primary Antibodies
Clone Name:	5C1
Applications:	WB
Recommended Dilution:	Western blot: 5 µg/ml. For details see protocol below.
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	His-tagged human Apaf-1 fusion protein corresponding to N-terminal amino acids (1-421 aa)
Specificity:	This antibody detects ~130 kDa of human Apaf-1 protein from whole cell lysate of human cell lines.
Formulation:	PBS containing 50% glycerol. Contains no preservatives. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein-A Sepharose
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	apoptotic peptidase activating factor 1
Database Link:	Entrez Gene 317 Human O14727

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Background: Apoptosis occurs during normal cellular development and involves dramatic changes in cellular structure. Disruption of apoptosis may contribute to cancer as well as other autoimmune diseases. Recently, the mammalian homologue of the key cell death gene CED-4 in C. elegans has been identified from human and mouse, and designated Apaf-1 (apoptosis protease activating factor 1) (Ref. 1,2). Apaf-1 binds to cytochrome c (Apaf-2), a reaction that requires dATP, and this leads to the formation of a large multimeric complex. The complex recruits pro-caspase-9, and activates caspase-9 (Apaf-3). Activated caspase-9 in turn cleaves and activates caspase-3, one of the proteases responsible for the proteolytic cleavage of many key proteins in apoptosis. Apaf-1 is ubiquitously expressed in human tissues (1).

Synonyms: Apaf-1, KIAA0413

Note: This product was originally produced by MBL International.

Protocol: SDS PAGE & Western Blotting
1) Rinse 1 × 10e7 of cells 3 times with PBS and suspend it with 1ml of 1 × Laemmli SDS-PAGE sample buffer, then lyse the cells by brief sonication (up to 20 seconds).
2) Boil the sample for 3-5 minutes and centrifuge at 12,000 × g for a minute. Other methods may be suitable.
3) Resolve 20 µ l of the sample per lane by 7.5 % SDS- polyacrylamide gel electrophoresis (See the manufacture's manual for electrophoresis conditions).
4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm 2 for 1 hour in a semi-dry transfer system. (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% Methanol). See the manufacture's manual for precise transfer procedure.
5) To reduce nonspecific binding, soak the membrane in Block Ace TM (Snow bland) for 1 hour at 37 o C, or overnight at 4 o C.
6) Dilute the primary antibody to 5 µ g/ml with Dilution Buffer (10% Block Ace TM /PBS). Incubate the membrane with primary antibody dilution for 1 hour at room temperature (20-25 o C). (The optimal concentration of antibody will depend on the experimental conditions.)
7) Wash the membrane with Wash Buffer (0.2% Tween20 in PBS) for 5-10 minutes × 3 times.
8) Incubate the membrane with secondary antibody (1:10,000 diluted horseradish peroxidase conjugated anti Mouse IgG goat antibody in Dilution Buffer) for 1 hour at room temperature.
9) Wash the membrane with Wash buffer (10 minutes × 3 times)
10) Wipe excess buffer from the membrane, then incubate it with appropriate chemiluminescence reagents for 1 minute. Remove excess reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to X-ray film in a dark room for 30 seconds. Develop the film as usual. The conditions for exposure and development may vary.