

Basic Information

Product Name	Anti-XAF1 Antibody
Gene Name	XAF1
Source	Rabbit
Clonality	Polyclonal
Isotype	IgG
Species Reactivity	human
Tested Application	WB, IHC, FCM, ELISA
Contents	500 ug/ml antibody with PBS, 0.02% NaN ₃ , 1 mg/ml BSA and 50% glycerol.
Immunogen	E.coli-derived human XAF1 recombinant protein (Position: Q197-S301).
Concentration	500 ug/ml
Purification	Immunogen affinity purified.
Observed MW	34 kDa
Dilution Ratios	Western blot (WB): 1:500-2000 Immunohistochemistry (IHC): 1:50-400 Flow Cytometry (Fixed): 1:50-200 Enzyme linked immunosorbent assay (ELISA): 1:100-1000 (Boiling the paraffin sections in 10mM citrate buffer, pH6.0, or PH8.0 EDTA repair liquid for 20 mins is required for the staining of formalin/paraffin sections.) Optimal working dilutions must be determined by end user.

Storage

12 months from date of receipt, -20°C as supplied. 6 months 2 to 8°C after reconstitution. Avoid repeated freezing and thawing.

Background Information

XAF1 also called XIAP-associated factor 1, antagonizes the anticaspase activity of XIAP and may be important in mediating apoptosis resistance in cancer cells. By genomic sequence analysis, the XAF1 gene contains 7 exons spanning 18 kb. Southern blot analysis suggested that XAF1 is a single-copy gene. The XAF1 gene is mapped on 17p13.1. Fluorescence microscopy demonstrated nuclear expression of XAF1, in contrast to the cytoplasmic expression of XIAP. Functional analysis indicated that XAF1 reverses the XIAP-mediated inhibition of CASP3 and its protection against apoptosis. Northern blot analysis detected wide expression of 3.9-, 4.5-, 6.0-, and 7.0-kb XAF1 transcripts, with highest levels in heart and ovary and lowest levels in brain and testis. Antisense experiments determined that loss of XAF1 expression enhances resistance to apoptosis. Alteration in XAF1 and XIAP RNA

expression levels may lead to increased apoptotic resistance and proliferation due to unregulated XIAP function in cancer cells.

Selected Validation Data

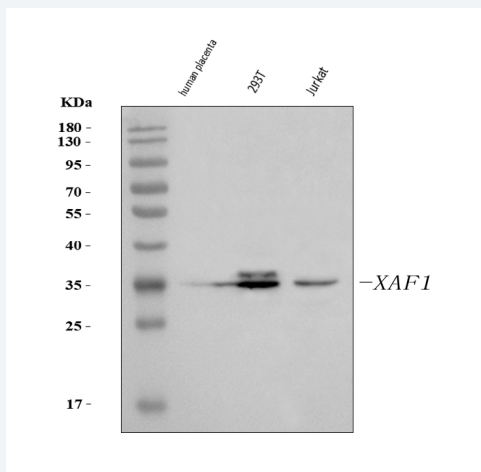


Figure 1. Western blot analysis of XAF1 using anti-XAF1 antibody (A03432-1). The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human placenta tissue lysates,

Lane 2: 293T whole cell lysates,

Lane 3: Jurkat whole cell lysates.

After electrophoresis, proteins were transferred to a membrane. Then the membrane was incubated with rabbit anti-XAF1 antigen affinity purified polyclonal antibody (A03432-1) at a dilution of 1:1000 and probed with a goat anti-rabbit IgG-HRP secondary antibody (Catalog # BA1054). The signal is developed using ECL Plus Western Blotting Substrate (Catalog # AR1197). A specific band was detected for XAF1 at approximately 34 kDa. The expected band size for XAF1 is at 35 kDa.

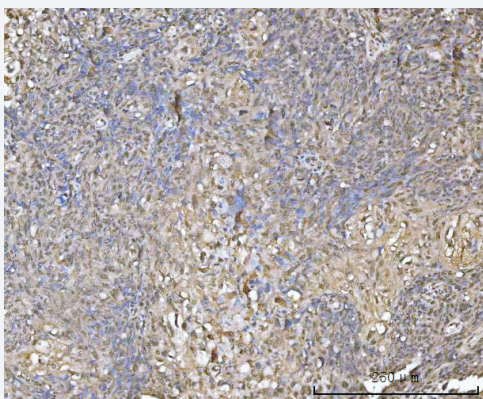


Figure 2. IHC analysis of XAF1 using anti-XAF1 antibody (A03432-1).

XAF1 was detected in a paraffin-embedded section of human lung cancer tissue. Biotinylated goat anti-rabbit IgG was used as secondary antibody. The tissue section was incubated with rabbit anti-XAF1 Antibody (A03432-1) at a dilution of 1:200 and developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB (Catalog # AR1022) as the chromogen.

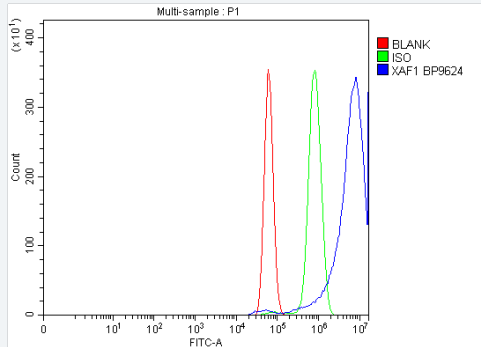


Figure 5. Flow Cytometry analysis of U87 cells using anti-XAF1 antibody (A03432-1).

Overlay histogram showing U87 cells stained with A03432-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-XAF1 Antibody (A03432-1) at 1:100 dilution for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG at 1:100 dilution used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.