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DIABLO Monoclonal Antibody (SMAC 17 1-87)

Catalog Number **MA1-936**

Product data sheet

Details	
Size	100 µg
Host/Isotope	Mouse / IgG1
Class	Monoclonal
Type	Antibody
Clone	SMAC 17 1-87
Immunogen	C-Terminal His tagged recombinant human Smac/DIABLO.
Conjugate	Unconjugated
Form	Liquid
Concentration	1 mg/mL
Purification	Protein G
Storage buffer	PBS with 1mg/mL BSA
Contains	0.05% sodium azide
Storage Conditions	-20° C, Avoid Freeze/Thaw Cycles

Species Reactivity	
Species reactivity	Human, Mouse, Rat
Published species	Rat, Not Applicable

Tested Applications	Dilution *
Immunohistochemistry (Paraffin) (IHC (P))	1:20-1:200
Immunoprecipitation (IP)	Assay-dependent
Western Blot (WB)	1 µg/mL
Immunocytochemistry (ICC/IF)	1:20-1:200

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

MA1-936 detects Smac/DIABLO from human, mouse and rat tissues. MA1-936 has been successfully used in Immunoprecipitation, Western blot, Immunohistochemistry (paraffin), Immunofluorescence and immunocytochemistry. By Western blot it detects a 25-kDa band representing Smac /DIABLO. MA1-936 antigen is C-term His tagged recombinant human Smac/DIABLO.

Background/Target Information

DIABLO (smac), a mitochondrial protein, activates various forms of apoptosis. This activation may be due to the neutralization of one or more members of the IAP family of apoptosis inhibitory proteins. Smac exits the mitochondria and enter the cytosol during certain apoptosis triggered events. Mitochondrial-mediated apoptosis is important in animal development and tissue homeostasis, with alterations resulting in a range of malignant disorders. Upon apoptotic stimuli, the mitochondrial proteins cytochrome c and Smac/DIABLO are released into the cytosol. The release of these proteins, however, occurs via different mechanisms. Smac/DIABLO has also been found to play a key role in regulating the sensitization of cancer cells to apoptosis (both immune and drug-induced).

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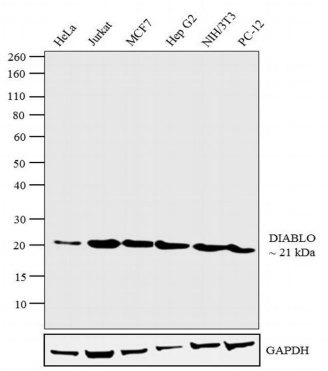
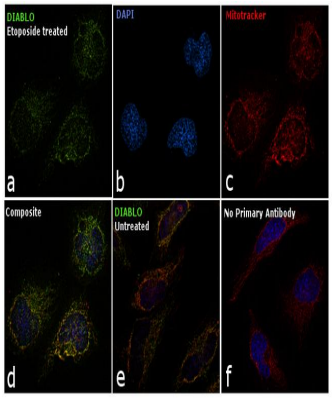
Product Images For DIABLO Monoclonal Antibody (SMAC 17 1-87)



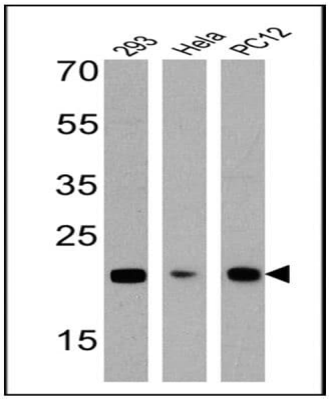
DIABLO Antibody (MA1-936) in WB

Western blot analysis was performed on membrane extracts (30 µg lysate) of HeLa (Lane 1), Jurkat (Lane 2), MCF7 (Lane 3), Hep G2 (Lane 4), NIH/3T3 (Lane 5) and PC-12 (Lane 6). The blot was probed with Anti-DIABLO Rabbit Polyclonal Antibody (Product # MA1-936, 1 µg/mL) and detected by chemiluminescence using Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25 µg/mL, 1:4000 dilution). A 21 kDa band corresponding to DIABLO was detected across the cell lines tested.



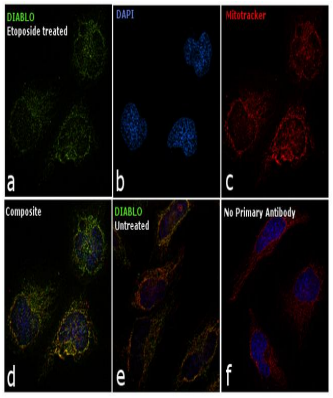
DIABLO Antibody (MA1-936)

Detection of altered subcellular localization of the target protein by cell treatment demonstrates antibody specificity. Immunofluorescence analysis of DIABLO using Mouse Monoclonal antibody (Product # MA1-936), shows change in localization of DIABLO from mitochondria to cytoplasm in Hela cells upon treatment with etoposide. {TM}



DIABLO Antibody (MA1-936) in WB

Western blot analysis of DIABLO was performed by loading 25 µg of 293 (Lane 1), Hela (Lane 2), and PC12 cell lysates (Lane 3) and a molecular weight protein ladder onto an SDS polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked with a blocking buffer at 4ºC overnight. The membrane was probed with a DIABLO monoclonal antibody (Product # MA1-936) at a dilution of 1:1000 overnight at 4°C, washed in TBST, and probed with an HRP-conjugated secondary antibody for 1 hr at room temperature in the dark. Chemiluminescent detection was performed using Pierce ECL Plus Western Blotting Substrate (Product # 32132). Results show a band at 21 kDa in all three cell lines.



DIABLO Antibody (MA1-936) in ICC/IF

Immunofluorescence analysis of DIABLO was performed using log phase HeLa cells treated with 25uM of Etoposide for 1 hour. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with DIABLO Mouse Monoclonal Antibody (Product # MA1-936) at 5 µg/mL in 0.1% BSA and incubated overnight at 4 degree and then labeled with Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing translocation of DIABLO from mitochondria to cytoplasm on treatment. Panel e represents untreated cells showing mitochondrial localization. Panel f represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.

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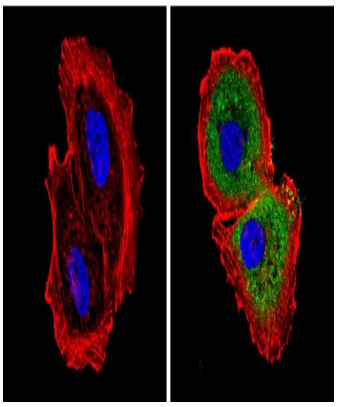
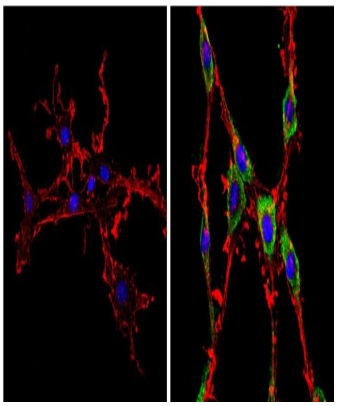


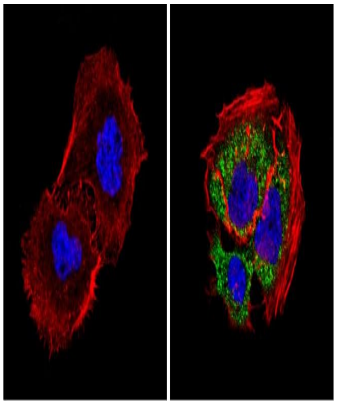
DIABLO Antibody (MA1-936) in ICC/IF

Immunofluorescent analysis of DIABLO (green) showing staining in the cytoplasm of MCF-7 cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with a DIABLO monoclonal antibody (Product # MA1-936) in 3% BSA-PBS at a dilution of 1:100 and incubated overnight at 4 ºC in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody in PBS at room temperature in the dark. F-actin (red) was stained with a fluorescent red phalloidin and nuclei (blue) were stained with Hoechst or DAPI. Images were taken at a magnification of 60x.



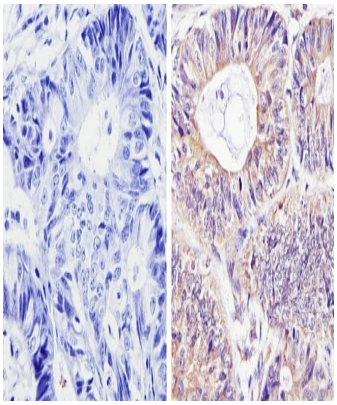
DIABLO Antibody (MA1-936) in ICC/IF

Immunofluorescent analysis of DIABLO (green) showing staining in the cytoplasm of PC12 cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with a DIABLO monoclonal antibody (Product # MA1-936) in 3% BSA-PBS at a dilution of 1:100 and incubated overnight at 4 ºC in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody in PBS at room temperature in the dark. F-actin (red) was stained with a fluorescent red phalloidin and nuclei (blue) were stained with Hoechst or DAPI. Images were taken at a magnification of 60x.



DIABLO Antibody (MA1-936) in ICC/IF

Immunofluorescent analysis of DIABLO (green) showing staining in the cytoplasm of A431 cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with a DIABLO monoclonal antibody (Product # MA1-936) in 3% BSA-PBS at a dilution of 1:100 and incubated overnight at 4 ºC in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody in PBS at room temperature in the dark. F-actin (red) was stained with a fluorescent red phalloidin and nuclei (blue) were stained with Hoechst or DAPI. Images were taken at a magnification of 60x.



DIABLO Antibody (MA1-936) in IHC (P)

Immunohistochemistry analysis of DIABLO showing staining in the cytoplasm of paraffin-treated human colon carcinoma (right) compared with a negative control in the absence of primary antibody (left). To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with a DIABLO monoclonal antibody (Product # MA1-936) diluted by 3% BSA-PBS at a dilution of 1:100 overnight at 4°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.

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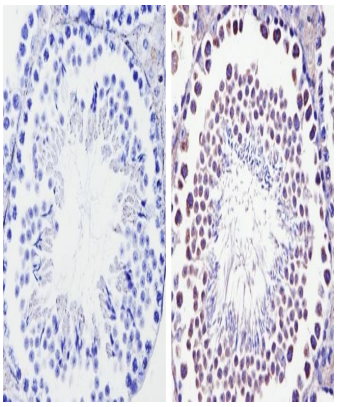
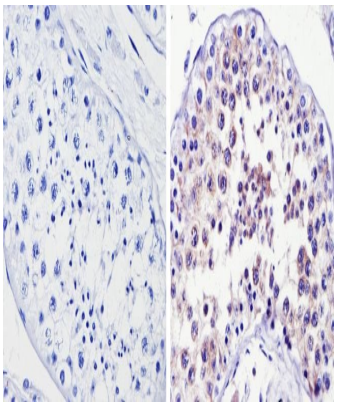


DIABLO Antibody (MA1-936) in IHC (P)

Immunohistochemistry analysis of DIABLO showing staining in the cytoplasm of paraffin-treated mouse testis tissue (right) compared with a negative control in the absence of primary antibody (left). To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with a DIABLO monoclonal antibody (Product # MA1-936) diluted by 3% BSA-PBS at a dilution of 1:100 overnight at 4°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.



DIABLO Antibody (MA1-936) in IHC (P)

Immunohistochemistry analysis of DIABLO showing staining in the cytoplasm of paraffin-treated human testis tissue (right) compared with a negative control in the absence of primary antibody (left). To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with a DIABLO monoclonal antibody (Product # MA1-936) diluted by 3% BSA-PBS at a dilution of 1:100 overnight at 4°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.

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