Smac Antibody
Cat. No.: 2409

Figure 1 Western Blot Validation in Human Heart Tissue Lysate
Loading: 15 μg of lysates per lane. Antibodies: Smac 2409 (1 μg/mL), 1h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

Figure 2 Independent Antibody Validation (IAV) via Protein Expression Profile in Cell Lines
Loading: 15 μg of lysates per lane. Antibodies: Smac 2409 (1 μg/mL), Smac 2411 (1 μg/mL), and beta-actin (1 μg/mL), 1h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

Figure 3 Western Blot Validation in Human, Mouse and Rat Cell Lines
Loading: 15 μg of lysates per lane. Antibodies: Smac 2409 (1 μg/mL), 1h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

Figure 4 Western Blot Validation in Mouse 3T3/NIH Cells
Loading: 15 μg of lysate. Antibodies: Smac 2409 (1 μg/mL), 1h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

Figure 5 Immunohistochemistry Validation of Smac in Human Ovary Tissue
Immunohistochemical analysis of paraffin-embedded Human Ovary tissue using anti-Smac antibody (2409) at 5 μg/ml. Tissue was fixed with formaldehyde and blocked with 10% serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4°C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.
Figure 6 KO Validation in Mouse Fibroblasts and Myoblasts (Ho et al., 2007)
The indicated MEFs or MEMs were exposed to 2 μM STS for 4 h and analyzed by Western blot. Accumulation of Smac/Diablo in mitochondria-depleted cytosol fractions from STS-treated Apaf-1 KO cells were detected by anti-smac antibodies. Smac expression was not detected in smac KO mice.

Figure 7 Immunohistochemistry Validation of Smac in Human Gastric Carcinoma (Kim et al., 2011)
Smac was highly expressed in gastric mucosa of patients with gastric carcinoma.

Figure 8 Immunofluorescence Analysis of Smac in NB4-LR1 Cells (Saumet et al., 2005)
NB4-LR1 cells were either treated with ATRA(1 μM) for 3 days without or with the T3C1 recombinant fragment (3 μM) or treated with staurosporine (STP, 5 μM) for 3.5 hours. STP, but not ATRA or AVRA/T3C1 induced the release of smac.

Figure 9 Induced Expression Validation in Rat Liver (Genestier et al., 2005)
Mitochondria from rat liver were treated with increasing concentrations of rPVL (A), rLukS (B), or Bax alpha (C) for 1 hour at 30°C. rPVL induces the release of the apoptogenic proteins cytochrome c and Smac/DIABLO from isolated mitochondria.

Figure 10 Overexpression Validation in HEK293T Cells (Flygare et al., 2012)
HEK293T cells were transiently transfected with Smac and Myc-tagged cIAP1, cIAP2, ML-IAP, or empty vector. Cells were lysed, and lysates were incubated with the indicated concentrations of 1 and immunoprecipitated with anti-Myc antibody (left panels). Samples were then immunoblotted with anti-Smac and anti-Myc antibodies. Whole-cell lysates are shown in the right panel.

Specifications

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<tr>
<th>HOST SPECIES:</th>
<th>Rabbit</th>
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<td>SPECIES REACTIVITY:</td>
<td>Human, Mouse, Rat</td>
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<td>IMMUNOGEN:</td>
<td>Anti-Smac antibody (2409) was raised against a peptide corresponding to 15 amino acids near the carboxy terminus of human Smac. The immunogen is located within the last 50 amino acids of Smac.</td>
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<td>TESTED APPLICATIONS:</td>
<td>ELISA, IF, IHC-P, IP, WB</td>
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### APPLICATIONS:

WB: 1 μg/mL; IHC: 5 μg/mL.

Antibody validated: Western Blot in human, mouse and rat samples; Immunohistochemistry in human samples. All other applications and species not yet tested.

### POSITIVE CONTROL:

1) Cat. No. 1301 - Human Heart Tissue Lysate
2) Cat. No. 1316 - Human Ovary Tissue Lysate
3) Cat. No. 11-201 - Human Ovary Tissue Slide

### PREDICTED MOLECULAR WEIGHT:

Predicted: 27kD

Observed: 20 kD

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### Advanced Validation

**Independent Antibody Validation in Cell lines** (Figure 2) shows similar Smac expression profile in human and mouse cell lines detected by two independent anti-Smac antibodies that recognize different epitopes, **2409** against human C-terminus domain and **2411** against murine C-terminus domain. Smac proteins are detected in the most tested cell lines at different expression levels by the two independent antibodies.

**KO Validation** (Figure 6): Anti-Smac antibody specificity was further verified by Smac knockout mice. Smac signal smac KO mice was disrupted as compared to control.

**Induced Expression Validation** (Figure 6,9): Smac expression detected by anit-Smac antibodies was up-regulated by STS or rPVL treatment.

**Overexpression Validation** (Figure 10): Smac overexpression was detected by anit-Smac antibodies on smac transfected HEK293T cells.

### ISOFORMS:

Human Smac has 3 isoforms, including isoform 1 (239aa, 27.1kD), isoform 2 (186aa, 21.2kD), and isoform 3 (195aa, 22.3kD). Mouse Smac has one isoform (237aa, 26.8kD) and Rat Smac also has one isoform (237aa, 26.9kD). 2409 can detect human, mouse and rat Smac.

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### Properties

**PURIFICATION:** Smac Antibody is affinity chromatography purified via peptide column.

**CLONALITY:** Polyclonal

**ISOTYPE:** IgG

**CONJUGATE:** Unconjugated

**PHYSICAL STATE:** Liquid

**BUFFER:** Smac Antibody is supplied in PBS containing 0.02% sodium azide.

**CONCENTRATION:** 1 mg/mL

**STORAGE CONDITIONS:** Smac antibody can be stored at 4°C for three months and -20°C, stable for up to one year. As with all antibodies care should be taken to avoid repeated freeze thaw cycles. Antibodies should not be exposed to prolonged high temperatures.
OFFICIAL SYMBOL: Diablo

ALTERNATE NAMES: Smac Antibody; Smac, AU040403, 0610041G12Rik, 1700006L01Rik, Smac, Diablo homolog, mitochondrial, Direct IAP-binding protein with low pI

ACCESSION NO.: NP_063940

PROTEIN GI NO.: 8953908

GENE ID: 66593

USER NOTE: Optimal dilutions for each application to be determined by the researcher.

Background and References

BACKGROUND: Smac Antibody: The inhibitor of apoptosis proteins (IAPs) regulate programmed cell death by inhibiting members of the caspase family of enzymes. A novel mammalian protein that binds to IAPs and neutralizes the inhibitory effect of IAPs on caspasess was recently identified and designated Smac/DIABLO. Smac/DIABLO is a mitochondrial protein that is released along with cytochrome c during apoptosis and activates cytochrome c/Apaf-1/caspase-9 pathway. Analysis of the structural basis of Smac/DIABLO reveals that the N-terminal amino acids are required for binding of Smac/DIABLO to IAPs and activation of caspasess. Smac/DIABLO is expressed in a variety of human and mouse tissues.

REFERENCES:

Citations

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<tr>
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<td>Watanabe et al. MITOPLD is a mitochondrial protein essential for nuage formation and piRNA biogenesis in the mouse germline. Dev Cell. 2011;20(3):364-75. PMID: 21397847</td>
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<td>15</td>
<td>Moubarak et al. FAIM-L is an IAP-binding protein that inhibits XIAP ubiquitinylation and protects from Fas-induced apoptosis. J Neurosci. 2013;33(49):19262-75. PMID: 24305822</td>
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