

## Certificate of Analysis

### Smac/DIABLO

(Recombinant protein expressed in *E.coli*)

Item # 23-057, 23-057-K, 23-057M

Parent Lot # D12SP005N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialing runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialing run.

**Product Description:** C-terminal cMyc and 6His-tagged, recombinant human Smac/DIABLO amino acids 56-end, expressed in *E.coli*. Purified using immobilized metal affinity chromatography.

Purity 94% by SDS-PAGE and Coomassie blue staining. MW = 23kDa.

**Formulation:** 3.012mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

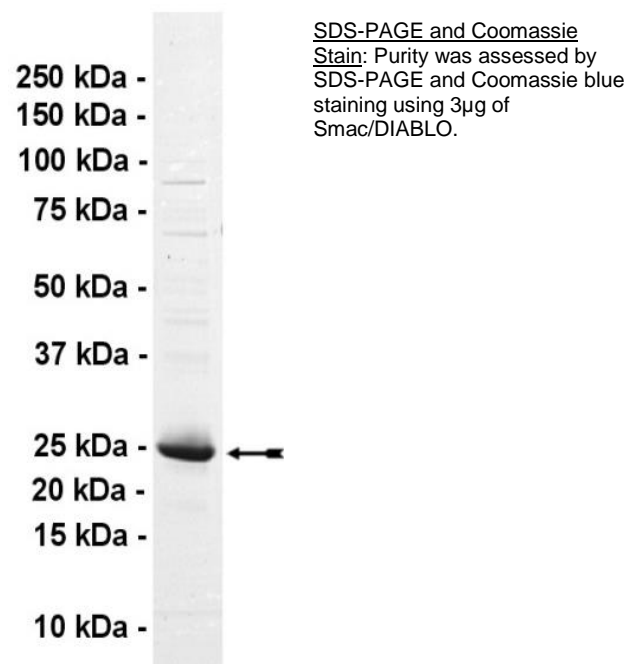
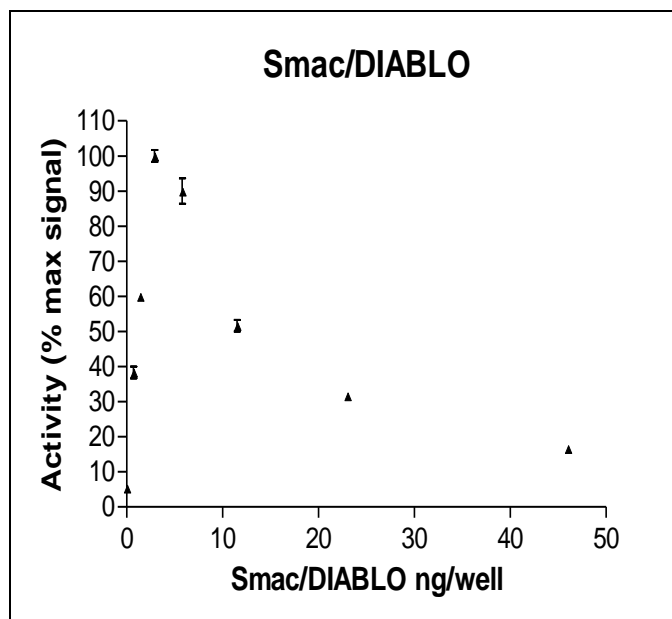
**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Assay:** This enzyme was titrated in a ubiquitination assay and the results normalised against the maximum signal.

**Protein Identity:** Confirmed identity as Smac/DIABLO by mass spectrometry.



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### Assay Protocol

#### Reagents:

- |                                  |                           |
|----------------------------------|---------------------------|
| 1. UBE1, active (Item # 23-021)  | 5. 1x Reaction Buffer     |
| 2. Ubch4, active (Item # 23-025) | 6. Biotinylated-Ubiquitin |
| 3. XIAP, active (Item # 23-056)  | 7. Stop Solution          |
| 4. Smac/DIABLO (Item # 23-057)   |                           |

#### Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH 7.5, 0.01% Tween 20, 5mM MgCl<sub>2</sub>).

Smac/DIABLO is incubated with 25mM MOPS pH 7.5, 0.01% Tween 20, 5mM MgCl<sub>2</sub>, 10μM ATP, 10nM UBE1, 50nM Ubch4, 0.01μg XIAP, and 2μM biotinylated-ubiquitin. The reaction is initiated with the addition of biotinylated-ubiquitin. After 30 minutes at room temperature the reaction is terminated by the addition of 25mM MOPS pH 7.5 containing 125mM EDTA, 150mM NaCl, and 0.05% Tween 20. Reaction products are separated by capture onto a microplate coated with anti-c-Myc antibody and washing with PBS containing 0.05% Tween 20. Ubiquitination of Smac/DIABLO is measured by detection of bound ubiquitin via electrochemiluminescence

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### Smac/DIABLO Information

<b><u>Protein</u></b>	human Smac/DIABLO
<b><u>Accession number</u></b>	GenBank NM_019887
<b><u>Alternative Names</u></b>	Diablo homolog, mitochondrial, Direct IAP-binding protein with low pI, Second mitochondria-derived activator of caspase (Smac)
<b><u>Key Facts</u></b>	Smac/DIABLO is a mitochondrial protein that is proteolytically processed and released during apoptosis along with cytochrome c and other pro-apoptotic factors. Once in the cytosol, Smac/DIABLO binds to inhibitors of apoptosis (IAP) proteins and disrupts the ability of the IAPs to inhibit caspases 3, 7, and 9 thereby promoting apoptosis. Along with several other pro-apoptotic proteins, Smac/DIABLO has been shown to be a substrate for the ubiquitin ligase XIAP.
<b><u>Related Products</u></b>	Item # 23-021 UBE1, active, Item # 23-025 Ubch4, active, Item # 23-056 XIAP, active

### **Selected References**

Du C. *et al.*, Smac, a Mitochondrial Protein that Promotes Cytochrome c-Dependent Caspase Activation by Eliminating IAP Inhibition. *Cell*, 102: 33-42, 2000

Verhagen A. M. *et al.*, Identification of DIABLO, a Mammalian Protein that Promotes Apoptosis by Binding to and Antagonizing IAP Proteins. *Cell*, 102: 43-53, 2000

MacFarlane M. *et al.*, Proteasome-mediated Degradation of Smac during Apoptosis: XIAP Promotes Smac Ubiquitination *in Vitro*. *J. Biol. Chem.* 277: 36611-36616, 2002

Morizane Y. *et al.*, X-Linked Inhibitor of Apoptosis Functions as Ubiquitin Ligase toward Mature Caspase-9 and Cytosolic Smac/DIABLO. *J. Biochem.* 137: 125-132, 2005

Reviewed and approved by site quality representative.

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