3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Smac/DIABLO
Human, Recombinant
Expressed in *E. coli*

Product Number **S 5941**

Product Description

Recombinant Human Smac/DIABLO encodes amino acid 56-239 of human Smac/DIABLO with a six histidine tag on the C-terminus. It is expressed in and purified from *Escherichia coli*. Amino acid sequence results indicate that the initiation methionine was removed in E. *coli*. Recombinant human Smac/DIABLO migrates as a polypeptide of 22 kDa on SDS-PAGE under reducing and non-reducing conditions. Size exclusion chromatography confirms that >95% of Smac/DIABLO is non-convalent dimer.

Apoptosis is a crucial process in development, normal cellular differentiation, and tissue homeostasis in all multicellular organisms.[1] Mitochondria play a key role in the commitment of cells to apoptosis. Release of cytochrome c from the mitochondria into the cytosol, in response to apoptosis signals, results in the activation of caspase 9 and the downstream caspases 3, 6, and 7. The bcl-2 family of proteins and the inhibitors of apoptosis proteins (IAPs), which include XIAP, c-IAP-1, and c-IAP-2 are major regulators of the mitochondria-based caspase activation pathway.

Smac/DIABLO (second-mitochondria-derived activator of caspase/direct IAP binding protein with low pI) is a mitochondria-derived pro-apoptotic protein that is released from the mitochondria in response to apoptotic stimuli.[2, 3] Smac/DIABLO promotes the caspase activity of the initiator caspase 9 and the effectors caspase 3 and caspase-7 by neutralizing the inhibitory activity of the IAPs, particularly of XIAP.[4-7] It promotes apoptosis by removing XIAP from the Apaf-1/caspase-9 apoptosome.[8, 9]

Smac/DIABLO exists as two isoforms, Smac/DIABLO-L and a short isoform Smac/DIABLO-S, differing in their N-terminal sequences. Smac/DIABLO-S does not bind IAPs, is inactive against caspase 9, and has low activity against caspase 3 and caspase 7. Smac/DIABLO-L contains an N-terminal signal sequence which is subsequently imported into the mitochondria.[2, 3]

During mitochondrial import, the amino-terminus of Smac/DIABLO-L is removed to generate the mature form of the molecule.[3, 4] The N-terminal AVPIA sequence in mature Smac/DIABLO is absolutely required for interaction with the BIR3 domain of XIAP. This sequence is substituted in Smac/DIABLO-S, indicating that the first five N-terminal residues of mature Smac/DIABLO are critical for its activity.[5-8] It has been suggested that Smac/DIABLO-S proapoptotic activity is due to a mechanism other than IAP binding.[10]

Mature Smac/DIABLO exists as a dimer formed through a hydrophobic interface within the N-terminal sequence. Mutations that disrupt Smac/DIABLO dimer formation abrogate the XIAP-neutralizing activity of this molecule, suggesting that Smac/DIABLO dimerization is essential for its proapoptotic activity.[5, 8]

Reagent

Recombinant Human Smac/DIABLO is supplied as a 0.2 µm filtered solution in 20 mM HEPES (pH 7.5) and 0.1 M KCl.

Concentration: Approximately 1.36 mg/ml

Storage/Stability

Freeze in working aliquots and store at –20 °C. This product may be stored for up to one week at 2-8 °C. Repeated freezing and thawing is not recommended. Do not store in a “frost-free” freezer.

Product Profile

Biological Activity: Reversal of XIAP and XIAP-Bir3 inhibition of the fluorescent caspase substrate DEVD-afc (N-Acetyl-Asp-Glu-Val-Asp-7-amido-4-trifluoro-methylcoumarin Product No. A 0466) cleavage activities in cell extracts (2 x 10^6 cells) activated by addition of cytochrome c and dATP.

The typical amount of Smac/DIABLO required to reverse XIAP (500 nM) inhibition of DEVD-afc cleavage by 50% in activated cell extracts is between 500 and 1500 nM.

The typical amount of Smac/DIABLO required to reverse XIAP-Bir3 (300 nM) inhibition of DEVD-afc cleavage by 50% in activated cell extracts is between 150 and 500 nM.

Purity: > 95% (SDS-PAGE, visualized by silver stain.

References

1. Vaux, D.L., and Korsmeyer, S.J., Cell, **96**, 245 (1999).
2. Du, C., et al., Cell, **102**, 33 (2000).
3. Verhagen, A.M., et al., Cell, **102**, 43 (2000).
4. Srinivasula, S.M., et al., J. Biol. Chem., **275**, 36152 (2000).
5. Chai, J., et al., Nature, **406**, 855 (2000).
6. Wu, G., et al., Nature, **408**, 1008 (2000).
7. Liu, Z., et al., Nature, **408**, 1004 (2000).
8. Srinivasula, S.M., et al., Nature, **410**, 112 (2001).
9. Ekert, P.G., et al., J. Cell Biol., **152**, 483 (2001).
10. Roberts, D.L., et al., J. Cell Biol., **153**, 221(2001).

kaa 01/03