REACTION BIOLOGY

PRODUCT DATASHEET

CIAP1 BIR2 domain (GST tag)

CATALOG NO.: APT-11-486 **LOT NO.:**

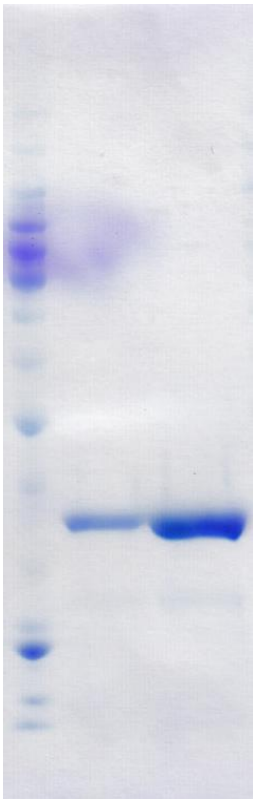
DESCRIPTION: Human recombinant BIR2 domain of CIAP1 (residues 173-256; NCBI Reference Sequence NM_001166.4; MW = 37.62 kDa) expressed in *E. coli* with an N-terminal GST-tag and C-terminal Strep-tag.

PURITY: >85% by SDS-PAGE

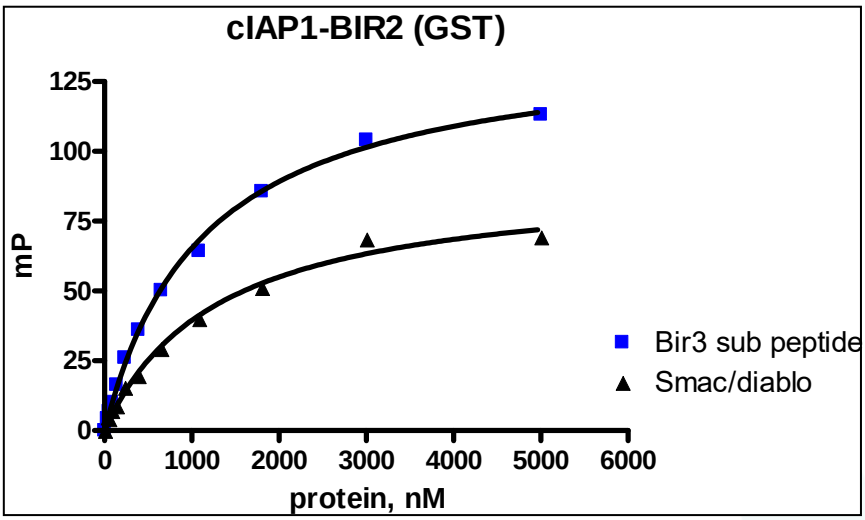
ASSAY CONDITIONS: RBC's cIAP1-BIR2 (GST) domain displays binding affinity for Smac/DIABLO and Bir3 substrate peptides. Reactions were performed with 25 nM FAM-labelled peptide at room temperature in 100mM Potassium Phosphate, pH 7.5, 0.1mg/mL BSA. The resulting polarization values (Ex. 480nm/Em. 535nm) was read following a 60 minute incubation. (See figure, below.)

SUPPLIED AS: ___ µg/µL in 50 mM Tris pH 7.5, 500 mM NaCl, 10% glycerol, 0.5 mM TCEP as determined by OD_{280}.

STORAGE: -70°C. Thaw quickly and store on ice before use. The remaining, unused, undiluted enzyme should be snap frozen, for example in a dry/ice ethanol bath or liquid nitrogen. Minimize freeze/thaws if possible, but very low volume aliquots (<5 µl) or storage of diluted enzyme is not recommended.



Coomassie blue-stained SDS-PAGE (4-12% acrylamide) of 4 and 10 µg of RBC cIAP1-BIR2 (GST). MW markers (left) are, from top, 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40, 30, 25, **20**, 15, 10 kDa.



cIAP2-BIR2 (GST) Binding Assay. FAM-Smac/DIABLO and Bir3 substrate peptide binding was detected using fluorescence polarization. The 20µL reaction contained 25nM peptide and variable concentration of cIAP1-BIR2 (GST). Polarization values (mP) were measured at an excitation wavelength at 480nm and an emission wavelength at 535nm using a ClarioStar reader (BMG Labtech) following a 60 minute incubation.

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