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Certificate of Analysis

XIAP, active

(Recombinant E3 ligase expressed in Sf21 insect cells)
Item # 23-056, 23-056-K, 23-056M
Parent Lot # D13AP009N

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

Product Description: *N*-terminal GST-tagged, recombinant human XIAP full length, expressed by baculovirus in Sf21 insect cells. Purified using glutathione sepharose, followed by gel filtration.
Purity 87% by SDS-PAGE and Coomassie blue staining. MW = 83kDa.

Activity (Parent lot# D13AP009N): This lot of XIAP is active and meets product specifications.

Formulation: 0.173mg/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.

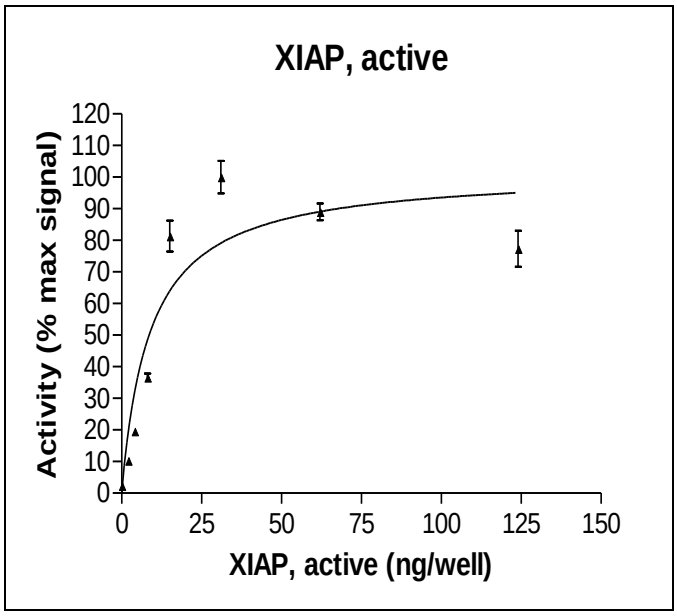
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

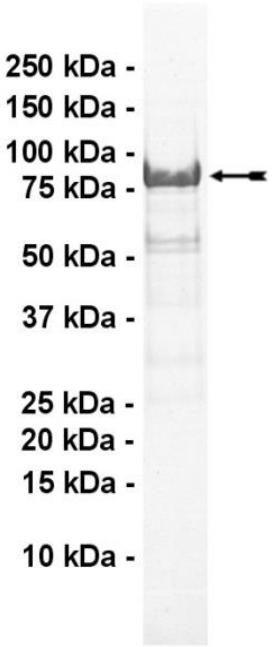
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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 XIAP by mass spectrometry.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of XIAP, active.

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E3 Assay Protocol

Reagents:

1. **UBE1, active (Item # 23-021)**
2. **UbcH4, active (Item # 23-025)**
3. **XIAP, active (Item # 23-056)**
4. **Smac/DIABLO (Item # 23-057)**
5. **1x Reaction Buffer**
6. **Biotinylated-Ubiquitin**
7. **Stop Solution**

Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH 7.5, 0.01% Tween 20, 5mM $MgCl_2$).

XIAP is incubated with 25mM MOPS pH 7.5, 0.01% Tween 20, 5mM $MgCl_2$, 10µM ATP, 10nM UBE1, 50nM UbcH4, 12.5nM substrate, 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. XIAP activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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XIAP Information

Protein human XIAP

Accession number GenBank NM_001167

Alternative Names Baculoviral IAP repeat-containing protein 4 (BIRC4), IAP-like protein, Inhibitor of apoptosis protein 3 (API3)

Key Facts X-linked inhibitor of apoptosis (XIAP) is one of a group of intracellular proteins which have been shown to act as endogenous inhibitors of caspases, the enzymatic effectors of apoptosis. XIAP possesses a *C*-terminal RING domain that functions as an E3 ubiquitin ligase and is able to ubiquitinate a variety of substrates thereby affecting a broad range of cellular activities beyond apoptotic suppression. There is evidence that XIAP plays a significant role in cell division, morphogenesis, heavy metal homeostasis, NF-kB activation and MAP kinase signalling. XIAP expression has been shown to be elevated in several malignancies and so is considered a promising target in anticancer therapies.

Related Products Item # 23-021 UBE1, active, Item # 23-025 UbcH4, active, Item # 23-057 Smac/DIABLO

Selected References

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Reviewed and approved by site quality representative.