Recombinant Human cIAP-2/HIAP-1

Catalog Number: 817-P2

DESCRIPTION

Source *E. coli*-derived

ATVID	10-His tag	SIEGRA	Human cIAP-2 (Asn2-Ser604) Accession # Q13489

N-terminus C-terminus

N-terminal Sequence Analysis Ala

Predicted Molecular Mass 71 kDa

SPECIFICATIONS

SDS-PAGE 73 kDa, reducing conditions

Activity Measured by its ability to inhibit DEVD-AFC cleavage activity in cell extracts activated by addition of cytochrome c and dATP.
The IC_{50} for this effect is 25-1000 nM.
Optimal dilutions should be determined by each laboratory for each application.

Purity >95%, by SDS-PAGE under reducing conditions and visualized by silver stain.

Formulation Supplied as a 0.2 µm filtered solution in HEPES, NaCl, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Jurkat E6 wild type cell extracts (see supplementary methods for preparation)
- Extraction Buffer: 50 mM HEPES, 10 mM KCl, 5 mM EGTA, 1 mM $MgCl_2$, 0.2% CHAPS, 0.2 mM DTT, pH 7.5
- Assay Buffer: 10 mM HEPES, 0.5 mM EGTA, 5 mM DTT, 10% Glycerol, pH 7.5
- Recombinant Human cIAP-2/HIAP-1 (rhcIAP-2) (Catalog # 817-P2)
- Cytochrome C, Bovine heart (Sigma, Catalog # C3131), 2 mg/mL stock in deionized water
- dATP (Sigma, Catalog # D6500), 10 mM stock adjusted to pH 7.5 with NaOH
- Cell Extracts from Jurkat E6 wild type cells (see above protocol)
- Substrate: Ac-Asp-Glu-Val-Asp-AFC (DEVD-AFC) (MP Biomedicals, Catalog # AFC138), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

Note: All reagents and assay components should be kept on ice until use.

1. Thaw cell extracts and centrifuge in a microcentrifuge at 14,000 rpms for 5 minutes at 4 °C. Transfer supernatants to chilled tubes and use within 1 hour.
2. Prepare a curve of rhcIAP-2 (MW: 71,000 Da) in Extraction Buffer. Make the following serial dilutions: 2500, 1500, 500, 250, 50, 25, and 5 nM. Note: High point may not be achievable depending on lot received.
3. Prepare the activator mixture by combining equal volumes of 2 mg/mL Cytochrome C and 10 mM dATP for working concentrations of 1 mg/mL and 5 mM, respectively.
4. Prepare reaction mixtures in tubes by combining 10 µL of each rhcIAP-2 curve dilution, 10 µL of cell extract supernatant, and 5 µL of the cytochrome C/dATP activator mixture. Also, prepare the following controls:
   a. Total Control: 10 µL of Extraction Buffer, 10 µL of cell extract supernatant, and 5 µL of the cytochrome C/dATP activator mixture.
   b. Inactive Control: 15 µL of Extraction Buffer and 10 µL of cell extract supernatant. The total reaction volume is 25 µL.
5. Incubate for 60 minutes at 30 °C.
6. After incubation, add 100 µL of Assay Buffer to each vial for a five-fold dilution. Mix briefly.
7. Dilute Substrate to 100 µM in Assay Buffer.
8. In a plate load 50 µL of diluted incubated reaction mixtures, and start the reaction by adding 50 µL of 100 µM Substrate.
9. Read at excitation and emission wavelengths of 400 nm and 505 nm, respectively, in kinetic mode for 5 minutes.
10. Derive the 50% inhibiting concentration (IC_{50}) of rhcIAP-2 by plotting normalized activity vs. reaction concentration of rhcIAP-2 (step 4) with Semi-Log Fitting [y = A + B * Log(x)]. Solve for x when y = 50.
11. Normalized activity may be determined using the following equation:

$$\% \text{ Normalized Activity} = \frac{\text{Sample (RFU/min)} - \text{Inactive Control}^{**} \text{ (RFU/min)}}{\text{Total Control (RFU/min)}} \times 100\%$$

Final Assay Conditions Per Reaction:

- rhcIAP-2 curve: 1000, 600, 200, 100, 20, 10, and 2 nM
- Substrate: 50 µM

PREPARATION AND STORAGE

Shipping The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**

- 6 months from date of receipt, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

cIAP-2 (also known as MIHC and HIAP-1) is a member of the inhibitor of apoptosis (IAP) family of proteins that inhibit the proteolytic activity of mature caspases. cIAP-2 has 3 BIR (baculovirus inhibitor of apoptosis) domains, a RING finger domain, and a caspase recruitment domain (CARD). cIAP-2 inhibits caspases through the direct interaction of its BIR domain with the active caspase. Caspase activity may be restored through interactions with the Reaper like motif on mitochondrial proteins such as SMAC/Diablo or HtrA2/Omi. cIAP-2 is reported to be cleaved by HtrA2/Omi.

References:

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