Recombinant Human Livin β/ML-IAP

Catalog Number: 787-LV

DESCRIPTION

Source	*E. coli*-derived Met1-Ser280, with a C-terminal 6-His tag Accession # AF311388
N-terminal Sequence Analysis	Met1
Predicted Molecular Mass	32 kDa

SPECIFICATIONS

SDS-PAGE	36 kDa, reducing conditions
Activity	Measured by its ability to reverse the inhibition of DEVD-AFC cleavage activity in cell extracts activated by addition of cytochrome c and dATP. The IC_{50} for this effect is typically 1.0-2.0 µM. See Activity Assay Protocol on www.RnDSystems.com **Optimal dilutions should be determined by each laboratory for each application.**
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
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 and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

Preparation of Cell Extracts

- Jurkat A3 wild type cells (ATCC # CRL-2570)
- Phosphate Buffered Saline (PBS, pH 7.4)
- Extraction Buffer: 50 mM HEPES-KOH (pH 7.5), 10 mM KCl, 5 mM EGTA, 1 mM $MgCl_2$, 0.2% CHAPS, 0.2 mM DTT
- Protease inhibitors: Cytochalasin B (Sigma # C6762), Chymostatin (Sigma # C7268), Leupeptin (Sigma # L8511), Antipain (Sigma # A6191), Pepstatin (Sigma # P4265), PMSF (Sigma # P7626)

Activation of Caspase in Cell Extracts

- 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 KOH
- Recombinant Human Livin β/ML-IAP(rhLivin β) (Catalog # 787-LV)
- Dilution Buffer: 25 mM HEPES (pH 7.5), 0.1 M NaCl, 1 mM DTT
- Assay Buffer: 25 mM HEPES (pH 7.5), 0.5 mM EGTA, 5 mM DTT, 10% Glycerol
- Substrate: Ac-Asp-Glu-Val-Asp-AFC (DEVD-AFC, MP Biomedicals, Catalog # AFC138) 500 µM stock in DMSO
- EIA/RIA 96-well plate (Costar, Catalog # 3369) or equivalent
- Fluorescence plate reader (Molecular Devices Model # SpectraMax Gemini EM) or equivalent

Assay

Preparation of Cell Extracts

1. Pellet cells from culture media by centrifugation at 1000 x g for 10 minutes at 4 °C.
2. Wash 2 times with PBS. Centrifudge as above and count cells before the final spin.
3. Add protease inhibitors to Extraction Buffer immediately prior to use. Final concentrations: 10 µg/mL Cytochalasin B, 2 ug/mL Chymostatin, 2 µg/mL Leupeptin, 2 µg/mL Antipain, 2 µg/mL Pepstatin, 100 µM PMSF and 1 mM DTT
4. Solubilize the cells in ice cold Extraction Buffer at a density of 2 x 10^8 cells/mL.
5. Thoroughly resuspend the pellet by gently pipetting up and down. Incubate on ice for 10 minutes.
6. Pipette 200 µL aliquots into chilled microcentrifuge tubes.
7. Snap freeze in liquid nitrogen and store at ≤-70 °C. (Note: Freeze immediately at ≤-70 °C if liquid nitrogen is unavailable to snap freeze)

Activation of Caspase in Cell Extracts

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

1. Thaw cell extracts and centrifuge at 14,000 x g for 5 minutes at 4 °C. Transfer supernatants to chilled tubes and use within 1 hour.
2. Dilute rhLivin β (MW: 32 kDa) to various concentrations in Dilution Buffer. Make an initial dilution series of: 25,000, 12,500, 5000, 2500, 1250, 250, 125 and 25 nM. The final concentration range will be 10,000 to 10 nM in 25 µl total reaction volume.
3. Add 10 µL of cell extract to a tube containing 2.5 µL Cytochrome c, 2.5 µL of dATP and 10 µL Livin β dilution.
4. Total (no Livin β) and inactive (no Livin β, Cytochrome-c, or dATP) controls should be run for each assay making up the volume difference with the appropriate buffer.
5. Incubate samples in a 30 °C water bath for 30 minutes.
6. To each well of a 96-well plate, add in the following order, 85 µL Assay Buffer and 5 µL of extracts activated in the presence or absence of added Livin β.
7. Start the reaction by adding 10 µL of 500 µM DEVD-AFC (50 µM final concentration).
8. Read at excitation and emission wavelengths 400 and 505 nm, respectively, in kinetic mode for 5 minutes.
9. Derive the 50% inhibiting concentration (IC_{50}) of rhLivin β by plotting RFU/min vs. concentration with 4-PL fitting.

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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, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

BACKGROUND

Livin β/ML-IAP is a member of the inhibitor of apoptosis (IAP) protein family, and contains a single BIR and RING finger motif. The anti-apoptotic protein appears to inhibit the activation of caspase-9 in cell extracts activated by cytochrome c and dATP. Livin β is the shorter of two splice variants, missing the first 54 base pairs from the 5' end of exon 6, encoding 18 amino acid residues in the BIR-RING linking region.

References:

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3. Ashhab, Y. *et al.* (2001) FEBS Lett. **495**:56.
4. Lin, J.-H. *et al.* (2000) Biochem. Biophys. Res. Commun. **279**:820.

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