Recombinant Human BID Caspase-8-cleaved

Catalog Number: 882-B8

DESCRIPTION

Source	*E. coli*-derived Met1-Asp195, protein was purified, cleaved by Caspase-8 and further purified Accession # P55957
N-terminal Sequence Analysis	Met1 (N-terminal fragment) & Gly61 (C-terminal fragment)
Predicted Molecular Mass	7 kDa (N-terminal fragment) & 15 kDa (C-terminal fragment)

SPECIFICATIONS

SDS-PAGE	9 kDa &14 kDa, reducing conditions
Activity	Measured by its ability to induce cytochrome c release from isolated mouse liver mitochondria using the Rat/Mouse Cytochrome c Quantikine ELISA (Catalog # MCTC0) to quantify cytochrome c. The typical EC_{50} for cytochrome c releasing activity is <50 nM. The EC_{50} for the desired application should be determined. Uncleaved Recombinant Human BID (Catalog # 846-BD) is available.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in HEPES-KOH and KCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Recombinant Human BID Caspase-8-cleaved (Catalog # 882-B8)
- Crude or enriched mouse liver mitochondria (See Preparation of mouse liver mitochondria at http://www.rndsystems.com/literature_cytochrome_c_release_assays_bcl-2.aspx)
- Dilution Buffer: 25 mM HEPES-KOH (pH 7.4), 0.1 M KCl, 1 mg/mL fatty acid free BSA* (Sigma, Catalog # A6003)
- Mitochondria Buffer: 125 mM KCl, 0.5 mM $MgCl_2$, 3.0 mM Succinic acid, 3.0 mM Glutamic acid, 10 mM HEPES-KOH (pH 7.4), 1 mg/mL BSA*, containing 25 µg/mL Leupeptin, 25 µg/mL Pepstatin, 3 µg/mL Aprotinin, 100 µM PMSF, and 10 µM Boc-Asp-FMK caspase inhibitor

**Note: Protease inhibitors and BSA should be added to the buffer immediately prior to use. BSA solution should be prepared at 100 mg/mL.*

Assay

Note: All buffers, proteins and tubes should be kept on ice. Assay volumes are 75 µL and are combined in 0.5 mL Eppendorf tubes.

1. Prepare dilutions of Recombinant Human BID Caspase-8-cleaved (MW: 22 kDa) in Dilution Buffer at concentrations of 500, 150, 50, 15, 5, 1.5, 0.5 and 0.15 nM. The final concentration range will be 100 to 0.03 nM in a total reaction volume of 75 µL.
2. Aliquot 15 µL of each of the BID dilutions to a series of tubes containg an additional 20 µL of Dilution Buffer and gently mix.
3. Initiate the reaction by adding 12 µL of mitochondria (approximately 25-30 µg) and 28 µL of Mitochondria Buffer containing protease inhibitors and BSA to each tube.
4. Two control samples must be run for each assay to determine the total amount of Cytochrome c that can be released from the mitochondria and the amount of spontaneously released Cytochrome c. Set up two samples containing only mitochondria and the appropriate buffers that have not been treated with any test proteins.
5. Cap the tubes and gently mix the contents for 5-10 seconds. Incubate in a 30 °C water bath for 30 minutes.
6. Total Cytochrome c in the assay should be determined by freezing the entire 75 µL rxn mix immediately after incubation at 30 °C.
7. Centrifuge the remaining samples at 16,000 x g for 5 min. at 2-8 °C. Remove and transfer a 50 µL aliquot of the supernatant to a new chilled tube. Samples may be analyzed immediately or stored at -20 °C in a manual defrost freezer.
8. Measure the levels of Cytochrome c in these samples using the Rat/Mouse Cytochrome c Quantikine® ELISA Kit (Catalog # MCTC0). See the Preparation of Samples for the Cytochrome c ELISA at http://www.rndsystems.com/literature_cytochrome_c_release_assays_bcl-2.aspx and additional instructions in the Rat/Mouse Cytochrome c Quantikine ELISA Kit product insert (Catalog # MCTC0).

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.

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BACKGROUND

BID is a 195 amino acid member of the Bcl-2 family of proteins that regulates outer mitochondrial membrane permeability (1). BID is a pro-apoptotic member that causes cytochrome c to be released from the mitochondria intermembrane space into the cytosol. In healthy cells BID is cytosolic. In response to Fas ligand or TNF, BID is cleaved by caspase-8 and it then relocates to the mitochondria outer membrane (2, 3). Cleavage of BID by caspase-8 generates a new N-terminus that contains a terminal glycine. It appears that the glycine is myristoylated and myristoylation serves to target BID to the mitochondria (4). BID may then interact with another pro-apoptotic Bcl-2 family member Bak (5). Interaction of BID with Bak causes altered mitochondrial membrane permeability. A 9-13 amino acid stretch called the BH3 region (Bcl-2 homology region) appears to mediate the BID interaction with other Bcl-2 family members. BID is neutralized by binding to the anti-apoptotic member Bcl-xL.

References:

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