Recombinant Human BIM_L Long (BIM L)

Catalog Number: 1325-BL

DESCRIPTION

Source	*E. coli*-derived human BIM Long (BIM L) protein Ala2-Arg120, with an N-terminal 6-His tag Accession # NP_006529
N-terminal Sequence Analysis	His
Predicted Molecular Mass	14.3 kDa

SPECIFICATIONS

SDS-PAGE	13-16 kDa and 30-35 kDa, reducing conditions
Activity	Measured by its ability to neutralize anti-apoptotic Bcl-2 family member inhibition of Recombinant Human BID Caspase-8-cleaved (Catalog # 882-B8)-mediated Cytochrome c release from isolated mitochondria. Release of Cytochrome c is quantified using the Rat/Mouse Cytochrome c Quantikine ELISA (Catalog # MCTC0). The typical EC_{50} value in this assay is 0.1-2 µM. **Optimal dose should be determined for each application.**
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in HEPES-KOH, DTT, KCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Recombinant Human BIM_L (Catalog # 1325-BL)
- Recombinant Human BID Caspase-8-cleaved (Catalog # 882-B8)
- Recombinant Human Bcl-w Minus C-Terminus (Catalog # 824-BW)
- 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)
- Formulation Buffer: 20 mM Tris (pH 8.5), 150 mM NaCl, 1 mM Dtt, 20% Glycerol, 1 mg/mL fatty acid free BSA* (Sigma, Catalog # A6003)
- BID Dilution Buffer: 25 mM HEPES-KOH (pH 7.4), 0.1 M KCl, 1 mg/mL fatty acid free BSA*
- 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 stock solution should be prepared at 100 mg/mL.

Assay

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

1. Prepare a stock solution of Recombinant Human Bcl-w Minus C-Terminus (MW: 19.6 kDa) in Formulation Buffer at 59 µg/mL. The final concentration will be 600 nM.
2. Prepare dilutions of Recombinant Human BIM_L (MW: 14.3 kDa) in Formulation Buffer at concentrations of 15,000, 5000, 1500, 500, 150, 50, and 15 nM. The final concentration range will be 3000 to 3 nM in a total rxn volume of 75 µL.
3. Aliquot 15 µL of each of the BIM_L dilutions to a series of tubes. Add 15 µL of the Recombinant Human Bcl-w Minus C-Terminus to each tube and gently mix. Incubate for 60 minutes at room temperature. Include one sample without Recombinant Human BIM_L (cleaved BID/Bcl-w control).
4. Prepare a stock solution of Recombinant Human BID Caspase-8-cleaved (MW: 22.0 kDa) in BID Dilution Buffer at 9.0 µg/mL. The final concentration will be 54 nM.
5. Add 10 µL of the Recombinant Human BID Caspase-8-cleaved Recombinant Human BID to each tube and gently mix. Incubate for 60 minutes at room temperature. Include one sample without Recombinant Human BIM_L or Bcl-w Minus C-Terminus (cleaved BID control).
6. Add 12 µL of mitochondria (approximately 25-30 µg) and 23 µL of Mitochondria Buffer with added protease inhibitors and BSA to each tube.
7. Two additional 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.
8. Cap the tubes and gently mix the contents for 5-10 seconds. Incubate in a 30 °C water bath for 30 minutes.
9. Total Cytochrome c in the assay should be determined by freezing the entire 75 µL rxn mix immediately after incubation at 30 °C.
10. Centrifuge the remaining samples at 16,000 x g for 5 minutes 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.
11. 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).

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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

BIM_L (also known as Bod) is one of several splice variants of BIM, a pro-apoptotic protein belonging to the BH3 domain-only subgroup of Bcl-2 family members. BIM_L is thought to promote apoptosis by binding and inhibiting the activity of anti-apoptoticBcl-2 family members, thereby inducing the release of cytochrome c from mitochondria. BIM_L is normally sequestered in an inactive conformation from anti-apoptotic Bcl-2 family members through binding to the microtubule-associated dynein motor complex.Certain apoptotic stimuli release BIM_L from microtubules to neutralize anti-apoptotic Bcl-2 family members, allowing for the initiation of apoptosis.

References:

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5. Marani, M. *et al.* (2002) Mol. Cell. Biol. **22**:3577.

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