Recombinant Human Bcl-2 related protein A1 Minus C-terminus

Catalog Number: 1160-A1

DESCRIPTION

Source	*E. coli*-derived Thr2-Ser152, with a C-terminal 6-His tag Accession # Q16548
N-terminal Sequence Analysis	Thr2
Structure / Form	Monomer
Predicted Molecular Mass	18 kDa

SPECIFICATIONS

SDS-PAGE	16 kDa, non-reducing conditions
Activity	Measured by its ability to inhibit Caspase-8-cleaved BID-mediated release of cytochrome c from isolated mouse liver mitochondria using the Rat/Mouse Cytochrome c Quantikine ELISA (Catalog # MCTC0) to quantify cytochrome c. The typical EC_{50} for human Bcl-2 related protein A1 Minus C-terminus in this assay is 15-200 nM.
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 NaH_2PO_4, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Recombinant Human Bcl-2 related protein A1 Minus C-terminus (Recombinant Human BCL2A1) (Catalog # 1160-A1)
- 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, 10% Glycerol, 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 a stock solution of Recombinant Human BID Caspase-8-cleaved (MW: 22 kDa) in Dilution Buffer at 9.0 µg/mL. The final concentration will be 54 nM.
2. Prepare dilutions of Recombinant Human BCL2A1 (MW: 18.3 kDa) in Dilution Buffer at concentrations of 9000, 3000, 900, 300, 90, 30, 9 and 3 nM. The final concentration range will be 3,000 to 1 nM in a total rxn volume of 75 µL.
3. Aliquot 25 µL of each of the BCL2A1 dilutions to a series of tubes. Add 10 µL of the Recombinant Human BID Caspase-8-cleaved to each tube and gently mix. Incubate for 60 min. at room temperature. Include one sample without Recombinant Human BCL2A1 (cleaved BID control).
4. Add 12 µL of mitochondria (approximately 25-30 µg) and 28 µL of Mitochondria Buffer containing protease inhibitors and BSA to each tube.
5. 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.
6. Cap the tubes and gently mix the contents for 5-10 seconds. Incubate in a 30 °C water bath for 30 min.
7. Total Cytochrome c in the assay should be determined by freezing the entire 75 µL rxn mix immediately after incubation at 30 °C.
8. 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.
9. 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

Bcl-2 related protein A1 (BCL2A1) is a member of the Bcl-2 family of proteins that regulate the outer mitochondrial membrane permeability. BCL2A1 is an anti-apoptotic member that prevents release of cytochrome c from the mitochondria intermembrane space into the cytosol. BCL2A1 is present on the outer mitochondrial membrane and is also found on other intracellular membranes in some cell types. Natural BCL2A1 contains a carboxyl-terminal mitochondria targeting sequence. Recombinant BCL2A1 is missing the mitochondrial targeting sequence but maintains its ability to neutralize pro-apoptotic Bcl-2 family members. Neutralization by BCL2A1 appears to occur by binding to the BH3 region of pro-apoptotic Bcl-2 family members. This activity does not require the mitochondrial targeting sequence.

References:

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3. Lin, Ey (1993) J. Immunol. **151**:197.
4. Karsan, A. (1996) Blood **87**:3089.

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