Recombinant Human
BLMH/Bleomycin Hydrolase
Catalog Number: 6200-CY

DESCRIPTION

Source  E. coli-derived
Ser2-Glu455, with an N-terminal Met and 6-His tag
Accession # Q13867

N-terminal Sequence Analysis  Met

Predicted Molecular Mass  53 kDa

SPECIFICATIONS

SDS-PAGE  45-50 kDa, reducing conditions

Activity  Measured by its ability to hydrolyze Met-AMC.
The specific activity is >500 pmol/min/μg, as measured under the described conditions.

Endotoxin Level  <1.0 EU per 1 μg of the protein by the LAL method.

Purity  >90%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 μg per lane.

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

Activity Assay Protocol

Materials
- Assay Buffer: 50 mM HEPES, 5 mM EDTA, 10 mM DTT, pH 7.0
- Recombinant Human BLMH/Bleomycin Hydrolase (rBLMH) (Catalog # 6200-CY)
- Substrate: Met-AMC (Bachem, Catalog # I-1265), 100 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay
1. Dilute rhBLMH to 1 μg/mL in Assay Buffer.
2. Incubate 1 μg/mL rhBLMH at 37 °C for 30 minutes.
3. Dilute Substrate to 2 mM in Assay Buffer.
4. Load 50 μL of the 1 μg/mL rhBLMH into a black well plate, and start the reaction by adding 50 μL of 2 mM Substrate. Include a Substrate Blank containing 50 μL Assay Buffer and 50 μL of 2 mM Substrate without any rhBLMH.
5. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
6. Calculate specific activity:

   \[
   \text{Specific Activity (pmoles/min/μg)} = \frac{\text{Adjusted } V_{\text{max}} \times \text{Conversion Factor} \times \text{amount of enzyme (μg)}}{\text{RFU/min}}
   \]

   *Adjusted for Substrate Blank

   **Derived using calibration standard 7-Amino, 4-Methyl Coumarin (AMC) (Sigma, Catalog # A-9891).

Final Assay Conditions Per Well:
- rhBLMH: 0.05 μg
- Substrate: 1 mM

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.

BACKGROUND

Bleomycin Hydrolase (BLMH) is a cysteine peptidase of the papain superfamily. It is named for its ability to hydrolyze the antitumor agent bleomycin and inactivate it (1). It has a papain-like catalytic triad (Cys-His-Asp) with optimum activity at neutral pH. In mammals it is expressed ubiquitously in all types of tissues and its expression is up-regulated in many tumors. It is present in the cytoplasm as homohexameric protein of approximately 300 kDa. In addition to its aminopeptidase activity, it has homocysteine-thiolactonase activity. The normal physiological function of BLMH is not clear. BLMH inactivates bleomycin, a glycopeptide anticancer agent, by deaminating it (2). BLMH has been suggested to play a role in the generation of MHC class I-presented peptides (3, 4). Diminished BLMH activity may contribute to the pathology of Alzheimer’s disease (AD) (5, 6). It is inhibited by cysteine protease inhibitors such as N-ethylmaleimide, iodoacetamide, para-hydroxymercuribenzoate, and E-64.

References:

PRODUCT SPECIFIC NOTICES

Coomassie is a registered trademark of Imperial Chemical Industries Ltd.

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