DESCRIPTION
Source
Mouse myeloma cell line, NS0-derived
Ser16-Tyr415, with a C-terminal 10-His tag
Accession # NP_083982
N-terminal Sequence Analysis Ser16
Predicted Molecular Mass 47 kDa

SPECIFICATIONS
SDS-PAGE 43 kDa and 48 kDa, reducing conditions
Activity Measured by its ability to cleave a colorimetric peptide substrate, Hippuryl-Arg. The specific activity is >5,000 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 >95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation Supplied as a 0.2 µm filtered solution in MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol
Materials
- Activation Buffer: 50 mM Tris, 10 mM CaCl₂, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Assay Buffer: 25 mM Tris, 0.1 M NaCl, pH 7.5
- Recombinant Mouse Carboxypeptidase B1/CPB1 (rmCPB1) (Catalog # 2898-ZN)
- Trypsin (Sigma, Catalog # T-1426)
- AEBSF (Catalog # E1001), 100 mM stock in DMSO
- Substrate: Hippuryl-Arg (Sigma, Catalog # H-2508), 25 mM in diH₂O
- 96-well Clear UV Plate (Coming, Catalog # 3635)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent
Assay
1. Activate rmCPB1 at 100 µg/mL with 1 µg/mL Trypsin in Activation Buffer.
2. Incubate at room temperature for 30 minutes.
3. Add AEBSF at a final concentration of 1 mM to stop reaction.
4. Dilute activated rmCPB1 to 2 µg/mL in Assay Buffer.
5. Dilute Substrate to 2 mM in Assay Buffer.
6. In a plate, load 50 µL of 2 µg/mL rmCPB1, and include a Substrate Blank containing 50 µL Assay Buffer.
7. Start the reaction by adding 50 µL of 2 mM Substrate into wells.
8. Read in kinetic mode for 5 minutes at an absorbance of 254 nm.
9. Calculate specific activity using the following formula:

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\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{\text{max}} \times (\text{OD/min}) \times \text{Conversion Factor}^{\text{**}} (\text{pmol/OD})}{\text{amount of enzyme (µg)}}
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*Adjusted for Substrate Blank
**Derived using calibration standard Hippuric acid (Sigma Catalog # 112003).

Final Assay Conditions Per Well:
- rmCPB1: 0.1 µg
- Substrate: 1 mM

PREPARATION AND STORAGE
Shipping
The product is shipped with polar packs. 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
Carboxypeptidase B1, encoded by the CPB1 gene, specifically cleaves the C-terminal Arg and Lys residues with a preference for Arg (1). The deduced amino acid sequence of mouse CPB1 consists of a signal peptide (residues 1 to 15), a pro region (residue 16 to 108), and a mature chain (residues 109 to 415). The purified rmCPB1 corresponds to the pro form, which can be activated by trypsin, the only pancreatic protease capable of generating active enzyme from the zymogen in vitro (1).

References: