Recombinant Human Pepsinogen A

Catalog Number: 6155-AS

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human Pepsinogen A protein Met1-Ala388, with a C-terminal 6-His tag Accession # P00790
N-terminal Sequence Analysis	Ile16 and Val63
Predicted Molecular Mass	41 kDa

SPECIFICATIONS

SDS-PAGE	40-43 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH_2 (Catalog # ES002). The specific activity is >7,000 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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 50 mM Sodium Citrate, pH 2.5
- Assay Buffer: 50 mM Sodium Citrate, 500 mM NaCl, pH 4.0
- Recombinant Human Pepsinogen A (rhPepsinogen A) (Catalog # 6155-AS)
- Substrate: MCA-Arg-Pro-Lys-Pro-Val-Glu-NVAL-Trp-Arg-Lys(DNP)-NH_2 (Catalog # ES002), 2 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 rhPepsinogen A to 100 µg/mL in Activation Buffer.
2. Incubate for 15 minutes at room temperature.
3. Dilute activated rhPepsinogen A to 0.02 µg/mL in Assay Buffer.
4. Dilute Substrate to 20 µM in Assay Buffer.
5. Load 50 µL of 0.02 µg/mL of rhPepsinogen A into a plate, and start the reaction by adding 50 µL of 20 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 20 µM Substrate.
6. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
7. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions

Per Well:

- rhPepsinogen A: 0.001 µg
- Substrate: 10 µM

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

Pepsins are aspartic proteases that are synthesized in the gastric mucosa and secreted into the stomach. They are released as zymogens called pepsinogens and then converted to active pepsins by the acidic pH of gastric juices (1). PGA-3, PGA-4, and PGA-5 are isozymogens of human Pepsinogen A, which differ in amino acid sequence by 2-4 residues (2). This recombinant human Pepsinogen A corresponds to PGA-4. Pepsins have optimal activity under conditions of acidic pH and are inhibited by pepstatin. Pepsin A has broad substrate specificity, but preferentially cleaves peptide bonds involving aromatic and aliphatic amino acids.

References:

1. Athauda, S.B. *et al.* (1989) J. Biochem. **106**:920.
2. Zwiers, A. *et al.* (1994) Clin. Nephrol. **41**:153.

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449