

**DESCRIPTION**

**Source** *E. coli*-derived  
Met1-His209, with a C-terminal 6-His tag  
Accession # Q9NXJ5

**N-terminal Sequence Analysis** Met1

**Predicted Molecular Mass** 24 kDa

**SPECIFICATIONS**

**SDS-PAGE** 24-26 kDa, reducing conditions

**Activity** Measured by the hydrolysis of pyroglutamyl-AMC.  
The specific activity is >9,500 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 Tris, 5 mM DTT, pH 9.0
  - Recombinant Human PGPEP-1 (rhPGPEP-1) (Catalog # 6278-CY)
  - Substrate: L-pyroglutamic acid-AMC (Pyr-AMC) (Bachem, Catalog # I-1300), 10 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 rhPGPEP-1 to 0.1 ng/μL in Assay Buffer.
  2. Dilute Substrate to 100 μM in Assay Buffer.
  3. Load into a plate 50 μL of 0.1 ng/μL rhPGPEP-1, and start the reaction by adding 50 μL of 100 μM Substrate. For Substrate Blanks, load 50 μL of Assay Buffer and 50 μL of 100 μM Substrate.
  4. Read plate at excitation and emission wavelengths of 380 nm and 460 nm, respectively, in kinetic mode for 5 minutes.
  5. Calculate specific activity:

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

\*Adjusted for Substrate Blank

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

- Final Assay Conditions** Per Well:
- rhPGPEP-1: 0.005 μg
  - Substrate: 50 μM

**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**

Pyroglutamyl peptidase-1 (PGPEP-1) is an omega peptidase which removes pyroglutamyl residues from the amino termini of peptides and proteins (1). It is a cytosolic cysteine peptidase that is expressed in most cell types (2). The enzyme requires a thiol-reducing agent for activity (3). PGPEP-1 is potentially involved in the inactivation of biologically active peptides that possess an amino terminal pyroglutamyl group (3). Examples of such peptides include neurotensin, luteinizing hormone releasing hormone, and thyrotropin-releasing hormone.

**References:**

1. Kilbane Z. *et al.* (2007) Mol. Cell. Biochem. **297**:189.
2. Cummins P.M. and B. O'Connor (1998) Biochim. Biophys. Acta. **1429**:1.
3. Dando P.M. *et al.* (2003) Protein Express. Purif. **28**:111.

**PRODUCT SPECIFIC NOTICES**

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