Recombinant Human Prolyl Oligopeptidase/PREP

Catalog Number: 4308-SE

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived Leu2-Pro710, with an N-terminal Met and 6-His tag Accession # P48147
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	81 kDa

SPECIFICATIONS

SDS-PAGE	73 kDa, reducing conditions
Activity	Measured by its ability to convert the substrate benzyloxycarbonyl-Gly-Pro-7-amido-4-methylcoumarin (Z-GP-AMC) to Z-Gly-Pro and 7-amino-4-methylcoumarin (AMC). The specific activity is >3,500 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	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in MES, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 250 mM NaCl, 2.5 mM DTT, pH 7.5
- Recombinant Human Prolyl Oligopeptidase/PREP (rhPREP) (Catalog # 4308-SE)
- Substrate: Z-Gly-Pro-AMC (Bachem, Catalog # I-1145)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhPREP to 0.1 µg/mL in Assay Buffer.
2. Dilute Substrate to 100 µM in Assay Buffer.
3. Incubate at room temperature for 5 minutes.
4. Load 50 µL of 0.1 µg/mL of rhPREP into a plate, and start the reaction by adding 50 µL of 100 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of Substrate.
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:

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

*Adjusted for Substrate Blank

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

Final Assay Conditions

Per Well:

- rhPREP: 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, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

BACKGROUND

Prolyl Oligopeptidase is a serine peptidase displaying specificity for the cleavage of Pro-Xaa bonds of oligopeptide substrates (1, 2). The peptidase is known to hydrolyze a variety of biologically active peptides such as bradykinin, substance P, neurotensin, and vasopressin (3). Because of its action on neuropeptides, Prolyl Oligopeptidase is considered to be involved in processes such as learning, memory, and depression (4).

References:

1. Tarrago, T. *et al.* (2005) J. Pept. Sci. **11**:283.
2. Yoshimoto, T. *et al.* (1977) Biochemistry. **16**:2942.
3. Wilk, S. (1983) Life Sci. **33**:2149.
4. Maes, M. *et al.* (1994) Biol. Psychiatry. **35**:545.

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