

TECHNICAL INFORMATION

ImmunO™

Catalog Number: 320821

Proline Specific Endopeptidase (From *Flavobacterium meningosepticum*)**Synonyms:** Prolyl endopeptidase; Post-proline cleaving enzyme; Post-proline endopeptidase**Description:** Proline specific endopeptidase, isolated from *Flavobacterium sp.*, cleaves specifically the peptide bonds on the carboxy side of proline residues^{1,2}. This enzyme is very close, in its properties, to a post-proline cleaving enzyme³⁻⁵. The substrates have been found to have the general structure Y-Pro-X, where Y is a peptide or N-protected amino acid and X may be an amino acid, peptide, amide or ester. Much slower hydrolysis is observed when the substrate is Y-Ala-X⁵.

Appearance: Lyophilized powder

Activity: Not less than 35U/mg protein

Contaminants (Typical): Aminopeptidase, Trypsin each less than 0.1%

Optimum pH: 7.0

Optimum temperature: 40°C

Isoelectric point: 9.1

Km value: 1.25 mM (Z-Gly-Pro-pNA)

Molecular weight: 78,000

Inhibitors: DFP, Z-Gly-Pro-CH₂Cl² (Z: carbobenzoxy-)**Assay for****Unit Definition:** Enzyme Activity: One unit is the enzyme activity which produces 1 µmol p-nitroaniline per minute at 30°C, pH 7.0, from Carbobenzoxy-Gly-Pro-pNA.**Reaction Mixture:**Buffer: 0.1 M phosphate buffer,
pH 7.01.0mlSubstrate: 2 mM Carbobenzoxy-Gly-Pro-pNA,
in 40% dioxane 0.25mlEnzyme: 0.05-0.2 u/ml in 50 mM
phosphate buffer, pH 7.00.1ml

Total volume 1.35ml

Procedure: Before adding the enzyme solution preincubation is carried out for 5 minutes at 30°C. After incubation for 10 minutes at 30°C, stop the reaction by adding 2.0 ml Triton X-100 solution (10g Triton X-100/95 ml 1M acetate buffer, pH 4.0). Prepare the blank by first mixing Triton X-100 solution with the mixed solution, followed by addition of the enzyme solution. Read absorbance of the test against the blank at 410 nm.

Calculation: Activity (U/ml) = $\frac{D A}{5.57 \times 10 \text{ (min)} \times 0.1 \text{ (ml)}} \times 3.35 \text{ (ml)} \times df$

D A: $A_{\text{test}} - A_{\text{blank}}$

5.57×10^3 : Molar extinction coefficient of p-nitroaniline

df: Dilution factor of enzyme solution

References:

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- Yoshimoto, T., Walter, R. and Tsuru, D., **J. Biol. Chem.**, 255, 4786, 1980.
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- Yoshimoto, T., Olowski, R.C. Walter, R., **Biochem.**, 16, 2942, 1977.
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Note: This product may contain a preservative such as sodium azide, thimerosal or proclin. Please see lot specific chemical credential for preservative information.