



MP Biomedicals, LLC.

29525 Fountain Parkway
Solon, Ohio 44139

Telephone: 440/337-1200

Toll Free: 800/854-0530

Fax: 440/337-1180

mailto:biotech@mpbio.com

web: www.mpbio.com

ImmunO™

Proline Specific Endopeptidase

Catalog #: 32082

Lot #: 5413K

Trivial Name: 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.

Specifications: Appearance Lyophilized powder

Activity Not less than 5.0 U/mg material

Contaminants 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

Storage: Stable for at least one year at 2-8°C in a desiccator

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 Z-Gly-Pro-pNA.

(Z: carbobenzoxy-)

Method:

Reaction Mixture

Buffer: 0.1 M phosphate buffer,

pH 7.0 1.0ml

Substrate: 2 mM Carbobenzoxy-Gly-Pro-pNA,

in 40% dioxane 0.25ml

Enzyme: 0.05-0.2 u/ml in 50 mM
phosphate buffer, pH 7.0 0.1ml

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 absorbancy of the test against the blank at 410 nm.

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

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

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

df: Dilution factor of enzyme solution

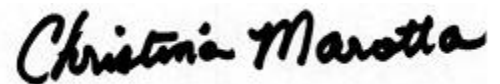
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5. Yoshimoto, T., Fischel, M., Olowski, R.C. and Walter, R., **J. Biol. Chem.**, 253, 3708, 1978.



Approved by: Christina Marotta
Quality Control Director

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