ELASTIN PRODUCTS COMPANY, INC
P.O. Box 568, Owensville, Missouri 65066 USA
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CERTIFICATE OF ANALYSIS

CARBOXYPEPTIDASE B
NO. CB276 (Lyophilized)

Lot No.: _____

CONCENTRATION.
A_{280} x 0.47 = mg protein per ml.

ACTIVITY.
One unit will hydrolyze 1 µmole of hippuryl-L-arginine per minute at pH 7.7 at 25°C.

_____125_____ Units CPB per mg of protein.

CONTAMINANTS.
Less than 1 unit per mg of protein of Carboxypeptidase A. Trypsin and chymotrypsin activities less than ___0.11_____%.

We hereby certify:

a) The Carboxypeptidase B is of animal origin only.
b) The Carboxypeptidase B was derived only from animals which were slaughtered in the United States in USDA inspected abattoirs where the animals were subjected to ante- and post mortem inspection and the animals were fit for human consumption or use in laboratory studies.
c) The United States if free from foot and mouth disease and rinderpest.

Signed :_____ Dated: _____

Reference: Folk, J. E., and Schirmer, E.W., J. Biol. Chem., 238, 3884 (1993).

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EC 3.4.7.2

SOURCE. Porcine Pancreas
SPECIFICATIONS.
Chromatographically purified, salt-free, lyophilized, soluble, white powder pH optimum 7.7 in 0.1 M Tris-HCl buffer containing 0.1 M NaCl. MW = 35000
Trypsin and chymotrypsin impurities removed by affinity chromatography.
Store at 5° C. Stable 1 – 2 years at -20° C.
SPECIFIC ACTIVITY.
130 – 150 units per mg of protein. One unit will hydrolyze 1.0 µM of hippuryl-L-arginine per minute at pH 7.7 at 25° C.

General Process.

Carboxypeptidase B (CPB) hydrolyzes the basic amino acids arginine and lysine from the C-terminal position of peptides.

Principle of Hydrolysis of Substrate.

Hippuryl-L-arginine + H_20 + CPB $\rightarrow$ Hippuric Acid + L-arginine + CPB.

Materials Required.

1. Tris buffer; 0.2 M pH 7.7 prepared at 25° C containing 0.2 M NaCl.
2. Substrate solution; 2.0 mM. Dissolve 68.0 mg hippuryl-L-arginine in 100 ml H_20. Heat to approximately 50° C with stirring to accelerate dissolution if necessary. Cool to room temperature. Prepare fresh daily. Keep in ice.
3. Enzyme solution; dissolve 0.1 mg/ml in tris buffer. Keep in ice.

Procedure.

1. Adjust the spectrophotometer to 254 nm and the cell temperature to 25° C.
2. As test solution mix, 1.5 ml tris-buffer and 1.5 ml substrate solution in the test cell. Equilibrate to 25° C.
3. As blank solution, mix 1.5 ml tris-buffer and 1.5 ml substrate. Likewise, equilibrate to 25° C.
4. At zero time add 20 µl CPB solution to the reaction solution, mix and determine the rate increase in absorbance vs. the blank, at 15 to 30 second intervals for 3 minutes. Calculate Δ_{254}nm/min from the initial linear reaction.

Calculation of Specific Activity.

$\in$, 1%, 280 = 21.2 mg/ml = Δ_{280} x 0.472 Vol = 3.02 ml

A= 254 nM T = 25° C Light Path = 1.0 cm

0.35 = increase in absorbency of 1 mM hippuric acid at pH 7.7

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$$\frac{\text{Units}}{\text{mg}} = \frac{(\Delta A/\text{min test} - \Delta A/\text{min Blank}) \; 3.02 \text{ ml}}{0.35 \times 0.002 \text{ mg}}$$

One unit will hydrolyze 1 µmole of hippuryl-L-arginine per minute at pH 7.7 and 25° C.

Assay Concentrations.

The 3.02 ml assay mixture contains 0.10 M tris, 1.0 mM Hippuryl-L-arginine, 0.10 M sodium chloride and 0.002 mg of CPB.

Assay of Possible Contaminants.

Note:
Usually the following assays are not necessary due to nil contamination by other pancreatic enzymes. These assays are given to assure quality of CPB.

Carboxypeptidase A
The substrate-buffer is 0.05 M Tris-HCl pH 7.5 containing 0.25 M sodium chloride and 1 mM hippuryl-L-phenylalanine. Use 0.25 mg of CPB in the assay at 25° C; reading Δ_{254} nm
$\in$, 1%, 280 = 19.2
One unit will hydrolyze 1.0 µmole of hippuryl-L-phenylanine per minute at pH 7.5 and 25° C.

Trypsin
The substrate-buffer is 0.10 M Tris-HCl pH 8.0 containing 0.05 M $CaCl_2$ + 1 mM N-benzoyl-L-arginine-4 nitroanilide-HCl (BAPNA). Use 0.50 mg of CPB in the assay at 25° C; read Δ_{410} nm.
$\in$ 1%, 280 nm = 14.3
One unit will hydrolyze 1 µmole of BAPNA per minute at pH 8.0 and 25° C

Chymotrypsin
The substrate buffer is 0.10 M Tris-HCl pH 8 containing 0.2 mM N-Succinyl-Ala-Ala-Pro-Phe-4 nitroanilide. Use 0.25 mg of CPB in the assay at 25°C; read Δ_{410} nm.
$\in$ 1%, 280 nm = 20.3

Note: To dissolve the lyophilized CPB; add the CPB to cold tris buffer, allow the mixture to sit - do not stir or agitate. After approximately 5 minutes aspirate the mixture along the inside of the vessel, do not introduce bubbles.