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## **CERTIFICATE OF ANALYSIS**

### **CARBOXYPEPTIDASE B NO. CB276 (Lyophilized)**

Lot No.: \_\_\_\_\_

#### **CONCENTRATION.**

$A_{280} \times 0.47 = \text{mg protein per ml.}$

#### **ACTIVITY.**

One unit will hydrolyze 1  $\mu\text{mole}$  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 is free from foot and mouth disease and rinderpest.

Signed : \_\_\_\_\_

Dated: \_\_\_\_\_



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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.

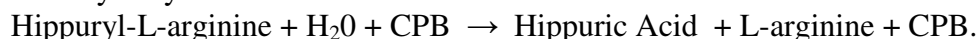
#### SPECIFIC ACTIVITY.

130 – 150 units per mg of protein. One unit will hydrolyze 1.0  $\mu$ M 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.



#### 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<sub>2</sub>O. 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  $\mu$ l 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  $\Delta_{254\text{nm}}/\text{min}$  from the initial linear reaction.

#### Calculation of Specific Activity.

$$\epsilon, 1\%, 280 = 21.2 \quad \text{mg/ml} = \Delta_{280} \times 0.472 \quad \text{Vol} = 3.02 \text{ ml}$$

$$A = 254 \text{ nM} \quad T = 25^\circ \text{ C} \quad \text{Light Path} = 1.0 \text{ cm}$$



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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  $\mu$ mole 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  $\Delta_{254} \text{ nm}$

$\in$ , 1%, 280 = 19.2

One unit will hydrolyze 1.0  $\mu$ mole of hippuryl-L-phenylalanine 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  $\text{CaCl}_2$  + 1 mM N-benzoyl-L-arginine-4 nitroanilide-HCl (BAPNA). Use 0.50 mg of CPB in the assay at 25° C; read  $\Delta_{410} \text{ nm}$ .

$\in$  1%, 280 nm = 14.3

One unit will hydrolyze 1  $\mu$ mole 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  $\Delta_{410} \text{ nm}$ .

$\in$  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.