3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of CATHEPSIN B
(EC 3.4.22.1)
Sigma Prod. No. C-6286

PRINCIPLE:

Ná-CBZ-L-Lysine p-Nitrophenyl Ester + H_2O $\xrightarrow{\text{Cathepsin B}}$ Ná-CBZ-L-Lysine + p-Nitrophenol

Abbreviation used:
CBZ = N-Carbobenzoxy

CONDITIONS: T = 25°C, pH = 5.0, A_{326nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 20 mM Sodium Acetate Buffer with 1.0 mM Ethylenediaminetetraacetic Acid and 5.0 mM L-Cysteine
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625, L-Cysteine, Hydrochloride, Monohydrate, Sigma Prod. No. C-7880, and Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 5.0 at 25°C with 1 M NaOH.)

B. Dimethyl Sulfoxide Solution (DMSO)
(Use Dimethyl Sulfoxide, Sigma Prod. No. D-5879.)

C. 5.2 mM Ná-CBZ-L-Lysine p-Nitrophenyl Ester Solution (Substrate)
(Prepare 2 ml in Reagent B using Ná-CBZ-L-Lysine p-Nitrophenyl Ester, Hydrochloride, Sigma Prod. No. C-3637.)

D. Cathepsin B Enzyme Solution
(Immediately before use, prepare a solution containing 2.5 - 5.0 units/ml of Cathepsin B in Reagent A.)

Enzymatic Assay of CATHEPSIN B
(EC 3.4.22.1)
Sigma Prod. No. C-6286

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reagent A (Buffer)	3.00	3.00
Reagent C (Substrate)	0.05	0.05

Mix by inversion and equilibrate to 25°C. Monitor the rate of increase in the absorbance at 326 nm for at least two minutes but no more than three minutes using a suitably thermostatted spectrophotometer. This rate should be approximately 0.03 absorbance units per minute. Then add:

Reagent D (Enzyme Solution)	0.01	------
Reagent A (Buffer)	------	0.01

Immediately mix by inversion and record the increase in A_{326nm} for approximately 3 minutes. Obtain the ΔA_{326nm}/minute using the maximum linear rate for both the Test and the Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{326nm}/\text{min Test} - \Delta A_{326nm}/\text{min Blank})(3.06)(df)}{(7.58)(0.01)}$$

3.06 = Total volume (in milliliters) of assay
df = Dilution factor
7.58 = Millimolar extinction coefficient of p-nitrophenol at 326 nm
0.01 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will hydrolyze 1 ìmole of Ná-CBZ-lysine p-nitrophenyl ester per minute at pH 5.0 at 25°C.

Enzymatic Assay of CATHEPSIN B
(EC 3.4.22.1)
Sigma Prod. No. C-6286

FINAL ASSAY CONCENTRATION:

In a 3.06 ml reaction mix, the final concentrations are 20 mM sodium acetate, 0.98 mM ethylenediaminetetraacetic acid, 4.9 mM L-cysteine, 0.08 mM Ná-CBZ-L-lysine p-nitrophenyl ester, 2% (v/v) dimethyl sulfoxide, and 0.025 - 0.050 unit cathepsin B.

REFERENCE:

Bajkowski, A.S. and Frankfater, A. (1975) *Analytical Biochemistry* **68**, 119-127

NOTES:

1. This assay is based on the cited reference.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.