SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of CHYMOTRYPSIN[1]
(EC 3.4.21.1)

PRINCIPLE:

BTEE + H_2O $\xrightarrow{\text{Chymotrypsin}}$ N-Benzoyl-L-Tyrosine + Ethanol

Abbreviation used:
BTEE = N-Benzoyl-L-Tyrosine Ethyl Ester

CONDITIONS: T = 25°C, pH = 7.8, A_{256nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 80 mM Tris HCl Buffer, pH 7.8 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.8 at 25°C with 1 M HCl.)

B. 1.18 mM N-Benzoyl-L-Tyrosine Ethyl Ester Solution (BTEE)
(Prepare 50 ml using N-Benzoyl-L-Tyrosine Ethyl Ester, Sigma Prod. No. B-6125, by initially dissolving in 31.7 ml of Methanol, Absolute, Sigma Stock No. 17-5. Add enough deionized water to make the final volume 50 ml.)

C. 2 M Calcium Chloride Solution ($CaCl_2$)
(Prepare 5 ml in deionized water using Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881.)

D. 1 mM Hydrochloric Acid Solution (HCl)
(Prepare 50 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)

E. Chymotrypsin Enzyme Solution
(Immediately before use, prepare a solution containing 2 - 5 units/ml of Chymotrypsin in cold Reagent D.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	1.42	1.42
Reagent B (BTEE)	1.40	1.40
Reagent C ($CaCl_2$)	0.08	0.08

Mix by inversion and equilibrate to 25°C. Monitor the A_{256nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (HCl)	------	0.10
Reagent E (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the increase in A_{256nm} for approximately 5 minutes. Obtain the ΔA_{256nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{256nm}\text{/min Test} - \Delta A_{256nm}\text{/min Blank})(3)(df)}{(0.964)\,(0.1)}$$

3 = Volume (in milliliters) of assay
df = Dilution factor
0.964 = Millimolar extinction coefficient of N-Benzoyl-L-Tyrosine at 256 nm.
0.1 = Volume (in milliliters) 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 1.0 μmole of BTEE per minute at pH 7.8 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 38 mM Tris, 0.55 mM N-benzoyl-L-tyrosine ethyl ester, 30% (v/v) methanol, 53 mM calcium chloride, 0.03 mM hydrochloric acid, and 0.2 - 0.5 unit chymotrypsin.

REFERENCES:

Wirnt, R. (1974) in *Methods of Enzymatic Analysis*, 2nd ed., Volume II, pp 1009-1012, Academic Press Inc., New York, NY

NOTES:

1. This assay procedure is not to be used to assay Chymotrypsin, Insoluble, Sigma Prod. Nos. C-9134 and C-7260, and Chymotrypsin, Acrylic Beads, Sigma Prod. No. C-5407.

2. This assay is based on the cited reference.

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