SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of ACYLASE I
(EC 3.5.1.14)
Sigma Prod. No. A-2156

PRINCIPLE:

N-Acetyl-L-Methionine + H_2O $\xrightarrow{\text{Acylase I}}$ L-Methionine + Acetic Acid

CONDITIONS: T = 37°C, pH = 8.0, A_{570nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 100 mM N-Acetyl-L-Methionine Solution, pH 8.0 at 37°C (NAMET)
(Prepare 25 ml by dissolving 478 mg of N-Acetyl-L-Methionine, Sigma Prod. No. A-3258 in 10 ml of deionized water and 2 ml of 1 M NaOH. Adjust to pH 8.0 at 25°C with 10 M NaOH and then bring up to a volume of 25 ml with deionized water.)

B. 200 mM Citrate Buffer, pH 5.0 at 37°C
(Prepare 200 ml in deionized water using Citric Acid, Free Acid, Anhydrous, Sigma Prod. No. C-0759. Adjust to pH 5.0 at 37°C with 1 M NaOH.)

C. 1.6% (w/v) Stannous Chloride Solution ($SnCl_2$)
(Prepare 12 ml in Reagent B using Stannous Chloride, Anhydrous, Sigma Prod. No. S-2752.)

D. Ethylene Glycol Monomethyl Ether
(Use Ethylene Glycol Monomethyl Ether, Sigma Prod. No. E-5378.)

E. 2% (w/v) Ninhydrin Color Reagent (NCR)
(Prepare 50 ml by dissolving 1 g of Ninhydrin, Sigma Prod. No. N-4876, in 25 ml of Reagent D. Then add 25 ml of Reagent B. Store in an amber colored bottle.)

F. 50% (v/v) 1-Propanol Solution
(Prepare 100 ml in deionized water using 1-Propanol, Sigma Stock No. 29,328-8.)

REAGENTS: (continued)

G. 0.5 mM Cobalt Chloride Solution ($CoCl_2$)
(Prepare 20 ml in deionized water using Cobalt Chloride, Hexahydrate, Sigma Prod. No. C-2644.)

H. 100 mM Barbital Buffer, pH 8.0 at 37°C
(Prepare 25 ml in deionized water using Barbital, Sodium Salt, Sigma Prod. No. B-0500. Adjust to pH 8.0 at 37°C with 1 M HCl.)

I. 0.8 mM L-Methionine Standard Solution (Std Soln)
(Prepare 20 ml in deionized water using L-Methionine, Sigma Prod. No. M-9625.)

J. Acylase I Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 unit/ml of Acylase I in cold deionized water.)

PROCEDURE:

Step 1:

Pipette (in milliliters) the following reagents into a suitable container:

	Test
Reagent J (Enzyme Solution)	1.00
Reagent H (Barbital Buffer)	2.00
Reagent G ($CoCl_2$)	1.00

Mix by swirling and equilibrate to 37°C. Then add:

Reagent A (NAMET)	1.00

Immediately mix by swirling and incubate at 37°C for exactly 30 minutes. Remove 1 ml from the Test and place into a glass-stoppered test tube. Heat in a boiling water bath for 3 minutes.

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PROCEDURE:

Step 2:

Pipette (in milliliters) the following reagents into a suitable container.

	Blank
Reagent J (Enzyme Solution)	1.00
Reagent H (Barbital Buffer)	2.00
Reagent G ($CoCl_2$)	1.00

Mix by swirling and equilibrate to 37°C. Then add:

Reagent A (NAMET)	1.00

Mix by swirling and immediately remove 1 ml from the Blank and place into a glass-stoppered test tube. Heat in a boiling water bath for 3 minutes.

Cool the Test and Blank solutions which have been boiled by using running water. Then add:

	Test	Test Blank	Std 1	Std 2	Std 3	Std 4	Std 5	Std Blank
Test Solution	1.00	----	----	----	----	----	----	----
Blank Solution	----	1.00	----	----	----	----	----	----
Reagent I (Std Soln)	----	----	0.10	0.20	0.40	0.60	1.00	----
Deionized Water	----	----	0.90	0.80	0.60	0.40	----	1.00
Reagent E (NCR)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Reagent C ($SnCl_2$)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10

Mix by swirling and place the vials in a boiling water bath for 20 minutes. Remove the vials and allow to cool to room temperature. Add 10 ml of Reagent F (1-Propanol) to each vial. Mix well and transfer the vial contents to suitable cuvettes. Determine the absorbance at 570 nm for each of the vials using a suitable spectrophotometer.

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CALCULATIONS:

Standard Curve:

ΔA_{570nm} Standard = A_{570nm} Standard - A_{570nm} Standard Blank

Prepare a standard curve by plotting the ΔA_{570nm} of the L-Methionine Standard Solution versus micromoles of L-Methionine.

Sample Determination:

ΔA_{570nm} Sample = A_{570nm} Test - A_{570nm} Sample Blank

Determine the µmoles of L-Methionine liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of L-Methionine liberated})(5)(df)}{(1)(1)}$$

5 = Total volume (in milliliters) of assay
df = Dilution factor
1 = Volume (in milliliter) of enzyme used
1 = Volume (in milliliter) of sample used in Colorimetric Determination

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

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of N-acetyl-L-methionine per hour at pH 8.0 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 5.00 ml reaction mix, the final concentrations are 40 mM barbital, 20 mM N-acetyl-L-methionine, 0.1 mM cobalt chloride, and 0.1 unit acylase I.

REFERENCE:

Moore, S. and Stein, W.H., (1948) *Journal of Biological Chemistry* **176**, 367-388

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NOTES:

1. This assay is based on the cited reference.

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