

**Enzymatic Assay of ACYLASE I'
(EC 3.5.1.14)
from Porcine**

PRINCIPLE:

N-Acetyl-L-Methionine + H₂O $\xrightarrow{\text{Acylase I}}$ L-Methionine + Acetic Acid

CONDITIONS: T = 25°C, pH = 7.0, A_{238nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Potassium Phosphate Buffer, pH 7.0 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.0 at 25°C with 1 M KOH.)
- B. 15 mM N-Acetyl-L-Methionine Solution (NAMet)
(Prepare 20 ml in Reagent A using N-Acetyl-L-Methionine, Sigma Prod. No. A-3258.)
- C. Acylase I Enzyme Solution
(Immediately before use, prepare a solution containing 1500 - 2500 units/ml of Acylase I in Reagent A. Let stand for 5-10 minutes at room temperature prior to assaying)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (NAMet)	2.90	2.90

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PROCEDURE: (continued)

Equilibrate to 25°C. Monitor the $A_{238\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	-----	0.10
Reagent C (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the decrease in $A_{238\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{238\text{nm}}/\text{minute}^2$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{238\text{nm}}/\text{min Test} - r A_{238\text{nm}}/\text{min Blank})(60)(3)(\text{df})}{(0.019)(0.1)}$$

60 = Conversion factor from minutes to one hour as per the Unit Definition

3 = Total Volume (in milliliters) of assay

df = Dilution factor

0.019 = Millimolar extinction coefficient³ of N-Acetyl-L-Methionine at 238 nm

0.1 = 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 1.0 μmole of N-acetyl-L-methionine per hour at pH 7.0 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 100 mM potassium phosphate, 14 mM N-acetyl-L-methionine and 150 - 250 units acylase I.

REFERENCE:

Mitz, M.A. and Schlueter, R.J. (1958) *Biochimica Et Biophysica Acta* **27**, 168-172

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NOTES:

1. This assay is not to be used to assay Acylase I, from *Aspergillus melleus*, Sigma Prod. No. A-2156.
2. The $\Delta A_{238\text{nm}}$ /minute should not be over 0.05.
3. This value was determined by Sigma.
4. This assay is based on the cited reference.
5. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.