Enzymatic Assay of ACYLASE I  
(EC 3.5.1.14)

PRINCIPLE:
N-Acetyl-L-Methionine + H₂O → L-Methionine + Acetic Acid

CONDITIONS:  T = 37°C, pH = 8.0, A₅₇₀nm, 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 (SnCl₂)  
(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.)
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REAGENTS:  (continued)

G. 0.5 mM Cobalt Chloride Solution (CoCl₂)  
(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  
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:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent J (Enzyme Solution)</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent H (Barbital Buffer)</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent G (CoCl₂)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent J (Enzyme Solution)</td>
</tr>
<tr>
<td>Reagent H (Barbital Buffer)</td>
</tr>
<tr>
<td>Reagent G (CoCl₂)</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Test Solution</th>
<th>Test Blank</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Std Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank Solution</td>
<td>----</td>
<td>1.00</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>----</td>
</tr>
<tr>
<td>Reagent I (Std Soln)</td>
<td>----</td>
<td>----</td>
<td>0.10</td>
<td>0.20</td>
<td>0.40</td>
<td>0.60</td>
<td>1.00</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>----</td>
<td>----</td>
<td>0.90</td>
<td>0.80</td>
<td>0.60</td>
<td>0.40</td>
<td>----</td>
</tr>
<tr>
<td>Reagent E (NCR)</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent C (SnCl₂)</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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:

\[ r \ A_{570\text{nm}}\text{ Standard} = A_{570\text{nm}}\text{ Standard} - A_{570\text{nm}}\text{ Standard Blank} \]

Prepare a standard curve by plotting the \( r \ A_{570\text{nm}}\) of the L-Methionine Standard Solution versus micromoles of L-Methionine.

Sample Determination:

\[ r \ A_{570\text{nm}}\text{ Sample} = A_{570\text{nm}}\text{ Test} - A_{570\text{nm}}\text{ Sample Blank} \]

Determine the \( \mu \)moles of L-Methionine liberated using the Standard curve.

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

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

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

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 \( \mu \)mole 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:

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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.

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