Enzymatic Assay of 3'-NUCLEOTIDASE
(EC 3.1.3.6)

PRINCIPLE:

\[
3'\text{-AMP} + H_2O \xrightarrow{3'\text{-Nucleotidase}} \text{Adenosine} + P_i
\]

Abbreviations used:
3'-AMP = Adenosine 3'-Monophosphate
P_i = Inorganic Phosphate

CONDITIONS: T = 37°C, pH = 7.5, \(A_{660nm}\), Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 100 mM Tris HCl Buffer, pH 7.5 at 37°C
   (Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.5 at 37°C with 1 M HCl.)

B. 40 mM Adenosine 3'-Monophosphate Solution (3'-AMP)
   (Prepare 5 ml in deionized water using Adenosine 3'-Monophosphate, Sodium Salt, Sigma Prod. No. A-0386.)

C. 20% (w/v) Trichloroacetic Acid Solution (TCA)
   (Prepare 25 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)

D. 10% (w/v) Ammonium Molybdate Solution
   (Prepare 25 ml in 10 N H_2SO_4 using Molybdic Acid, Ammonium Tetrahydrate Salt, Sigma Prod. No. M-0878.)

E. Taussky-Shorr Color Reagent (TSCR)
   (Prepare 100 ml by adding 10 ml of Reagent D to 70 ml of deionized water. Add 5 g Ferrous Sulfate Heptahydrate, Sigma Prod. No. F-0131 and mix until dissolved. Add enough deionized water for a final volume of 100 ml. Store in an amber container.)
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REAGENTS: (continued)

F. Phosphorus Standard Solution (P Std)  
(Use Phosphorus Standard Solution, Sigma Stock No. 661-9. The phosphorus concentration is 20 µg/ml, 0.645 µmoles/ml.)

G. 3'-Nucleotidase Enzyme Solution  
(Immediately before use, prepare a solution containing 1.5 - 2.5 units/ml of 3'-Nucleotidase in cold Reagent A.)

PROCEDURE:

Step 1:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.85</td>
<td>1.85</td>
</tr>
<tr>
<td>Reagent B (3'-AMP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

| Reagent G (Enzyme Solution) | 0.05 | ------ |

Immediately mix by swirling and incubate at 37°C for exactly 15 minutes. Then add:

| Reagent C (TCA)  | 0.20 | 0.20   |
| Reagent G (Enzyme Solution) | ------ | 0.05   |

Mix by swirling and centrifuge to clarify.

Step 2:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (TSCR)</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>Test Supernatant (from Step 1)</td>
<td>0.30</td>
<td>------</td>
</tr>
<tr>
<td>Blank Supernatant (from Step 1)</td>
<td>------</td>
<td>0.30</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>1.20</td>
<td>1.20</td>
</tr>
</tbody>
</table>
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PROCEDURE:

Mix by swirling and incubate at 25°C for 10 minutes. Transfer the solutions to suitable cuvettes and record the $A_{660\text{nm}}$ for both the Test and Blank using a suitable spectrophotometer.

COLORIMETRIC ASSAY:

Standard Curve:

A standard curve is made by pipetting (in milliliters) the following reagents into suitable containers:

<table>
<thead>
<tr>
<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>Reagent F (P Std) 0.10</td>
<td>0.25</td>
<td>0.35</td>
<td>0.60</td>
<td>1.00</td>
<td>---</td>
</tr>
<tr>
<td>Deionized Water 1.40</td>
<td>1.25</td>
<td>1.15</td>
<td>0.90</td>
<td>0.50</td>
<td>1.50</td>
</tr>
<tr>
<td>Reagent E (TSCR) 1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Mix by inversion and incubate at 25°C for 10 minutes. Transfer the solutions to suitable cuvettes and record the $A_{660\text{nm}}$ for the Standards and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

$\Delta A_{660\text{nm}}$ Standard = $A_{660\text{nm}}$ Standard - $A_{660\text{nm}}$ Standard Blank

Prepare a Standard curve by plotting the $\Delta A_{660\text{nm}}$ of the Standard vs µmoles of Phosphate

Sample Determination:

$\Delta A_{660\text{nm}}$ Test = $A_{660\text{nm}}$ Test - $A_{660\text{nm}}$ Test Blank

Determine the µmoles of Phosphate liberated using the Standard curve.

Units/ml enzyme = $\frac{\text{µmoles of Phosphate released}}{(2.2)(df)} (0.05)(15)$

2.2 = Total volume (in milliliters) of Step 1
df = Dilution factor
0.05 = Volume (in milliliter) of enzyme used
15 = Time (in minutes) of assay as per the Unit Definition
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UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of inorganic phosphorus from adenosine 3'-monophosphate per minute at pH 7.5 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 2.0 ml reaction mix the final concentrations are 95 mM Tris, 2 mM adenosine 3'-monophosphate, and 0.075 – 0.125 unit 3'-nucleotidase.

REFERENCE:


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.