Enzymatic Assay of GABASE

PRINCIPLE:

\[
\gamma\text{-Amino-}n\text{-Butyrate} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{GABGT}} \text{Succinic Semialdehyde} + \text{Glutamate}
\]

\[
\text{Succinic Semialdehyde} + \beta\text{-NADP} \xrightarrow{\text{SSDH}} \text{Succinate} + \text{NADPH}
\]

Abbreviations used:

GABGT = \gamma\text{-Aminobutyric Glutamic Transaminase}
β-NADP = \beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form}
SSDH = Succinic Semialdehyde Dehydrogenase
β-NADPH = \beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form}

CONDITIONS: T = 25°C, pH = 8.6, \text{A}_{340nm}, \text{Light path} = 1 \text{ cm}

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Pyrophosphate Buffer, pH 8.6 at 25°C
(Prepare 100 ml in deionized water using Pyrophosphate, Tetrapotassium, Anhydrous, Adjust to pH 8.6 at 25°C with 1 M HCl.)

B. 100 mM 2-Mercaptoethanol Solution (2-ME)
(Prepare 10 ml in deionized water using 2-Mercaptoethanol.)

C. 25 mM \beta\text{-Nicotinamide Adenine Dinucleotide Phosphate Solution (β-NADP)}
(Dissolve the contents of one 30 mg vial of \beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Preweighed Vial, in the appropriate volume of deionized water. Neutralize to pH 7.0 with solid Sodium Bicarbonate)}

D. 60 mM \gamma\text{-Amino-}n\text{-Butyric Acid Solution (GABA)}
(Prepare 10 ml in Reagent A using \gamma\text{-Amino-}n\text{-Butyric Acid.})
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REAGENTS:

E. 100 mM a-Ketoglutarate Solution (a-Ketoglut)  
(Prepare 2 ml in deionized water using a-Ketoglutaric Acid, Monosodium Salt.)

F. 75 mM Potassium Phosphate Buffer with 25% (v/v) Glycerol, pH 7.2 at 25°C (Enz Dil)  
(Prepare 25 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, and Glycerol, Adjust to pH 7.2 at 25°C with 1 M KOH.)

G. Gabase Enzyme Solution  
(Immediately before use, prepare a solution containing 1 - 2 units/ml of Gabase in cold Reagent F.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.30</td>
<td>2.30</td>
</tr>
<tr>
<td>Reagent B (2-ME)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent E (a-Ketoglut)</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Reagent C (ß-NADP)</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Reagent D (GABA)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the \(A_{340\text{nm}}\) until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent G (Enzyme Solution)</td>
<td>0.02</td>
<td>------</td>
</tr>
<tr>
<td>Reagent F (Enz Dil)</td>
<td>------</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in \(A_{340\text{nm}}\) for approximately 5 minutes. Obtain the \(r_{A_{340\text{nm}}/\text{minute}}\) using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r_{A_{340\text{nm}}/\text{min Test}} - r_{A_{340\text{nm}}/\text{min Blank}})(3.02)(\text{df})}{(6.22)(0.02)}
\]

3.02 = Total volume (in milliliters) of assay  
\(\text{df} = \text{Dilution factor}\)
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CALCULATIONS: (continued)

6.22 = Millimolar extinction coefficient of ß-NADPH at 340nm
0.02 = 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 convert 1.0 µmole of ß-aminobutyric acid (GABA) to succinic semialdehyde and then to succinate per minute with a stoichiometric reduction of 1.0 µmole of NADP⁺ at pH 8.6 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.02 ml reaction mix, the final concentrations are 86 mM potassium pyrophosphate, 3.3 mM 2-mercaptoethanol, 1.2 mM ß-nicotinamide adenine dinucleotide phosphate, 5 mM a-ketoglutarate, 6.0 mM ß-amino-n-butyric acid, 0.50 mM potassium phosphate, 0.17% (v/v) glycerol, and 0.02-0.04 unit gabase.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.

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

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