Enzymatic Assay of GABASE

PRINCIPLE:

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

$$\text{Succinic Semialdehyde} + \text{ß-NADP} \xrightarrow{\text{SSDH}} \text{Succinate} + \text{NADPH}$$

Abbreviations used:
GABGT = γ-Aminobutyric Glutamic Transaminase
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
SSDH = Succinic Semialdehyde Dehydrogenase
ß-NADPH = ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

CONDITIONS: T = 25°C, pH = 8.6, A_{340nm}, Light path = 1 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, Sigma Prod. No. P-8260. 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, Sigma Prod. No. M-6250.)

C. 25 mM ß-Nicotinamide Adenine Dinucleotide Phosphate Solution (ß-NADP)
(Dissolve the contents of one 30 mg vial of ß-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Preweighed Vial, Sigma Stock No. 240-330 in the appropriate volume of deionized water. Neutralize to pH 7.0 with solid Sodium Bicarbonate, Sigma Prod. No. S-8875.)

D. 60 mM γ-Amino-n-Butyric Acid Solution (GABA)
(Prepare 10 ml in Reagent A using γ-Amino-n-Butyric Acid, Sigma Prod. No. A-2129.)

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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, Sigma Prod. No. K-1875.)

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, Sigma Prod. No. P-5379, and Glycerol, Sigma Prod. No. G-9012. 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:

	Test	Blank
Reagent A (Buffer)	2.30	2.30
Reagent B (2-ME)	0.10	0.10
Reagent E (a-Ketoglut)	0.15	0.15
Reagent C (ß-NADP)	0.15	0.15
Reagent D (GABA)	0.30	0.30

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

Reagent G (Enzyme Solution)	0.02	------
Reagent F (Enz Dil)	------	0.02

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

CALCULATIONS:

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

3.02 = Total volume (in milliliters) of assay
df = Dilution factor

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 ?-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 γ-amino-n-butyric acid, 0.50 mM potassium phosphate, 0.17% (v/v) glycerol, and 0.02-0.04 unit gabase.

REFERENCE:

Jakoby, W.B. (1962) *Methods in Enzymology*, V, 771-774

NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.