

**Enzymatic Assay of GLUTAMINASE
(EC 3.5.1.2)**

PRINCIPLE:

Glutamine + H₂O $\xrightarrow{\text{Glutaminase}}$ Glutamic Acid + NH₃

Glutamic Acid + β-NAD $\xrightarrow{\text{GDH}}$ 2-Ketoglutarate + β-NADH + NH₃

Abbreviations used:

β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form

GDH = Glutamic Dehydrogenase

β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 37°C, pH = 8.6, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 206 mM Potassium Phosphate and 63 mM Tris HCl Buffer with 0.25 mM Ethylenediaminetetraacetic Acid, pH 8.6 at 37°C (Phos/Tris)
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504, Trizma Base, Prod. No. T-1503, and Ethylenediaminetetraacetic Acid, Sigma Stock No. ED4S. Adjust to pH 8.6 at 37°C with 1 M HCl.)
- B. 100 mM L-Glutamine Solution (Glutamine)
(Prepare 10 ml in deionized water using L-Glutamine, Sigma Prod. No. G-3126. Store on ice. **PREPARE FRESH.**)
- C. 3000 mM Hydrochloric Acid Solution (HCl)
(Prepare 30 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020).
- D. 400 mM Potassium Phosphate and 10 mM Boric Acid Buffer, pH 8.0 at 37°C (Phos/Boric Acid)
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Prod. No. P-5504 and Boric Acid, Prod. No. B-0252. Adjust to pH 8.0 at 37°C with 1 M H₃PO₄.)

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REAGENTS: (continued)

- E. 130 mM Tris HCl Buffer, pH 9.4 at 37°C (Tris/HCl)
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 9.4 at 37°C with 1 M HCl.)
- F. 20 mM β -Nicotinamide Adenine Dinucleotide Solution (β -NAD)
(Prepare 10 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-1636.)
- G. 20 mM Adenosine 5'-Diphosphate Solution (ADP)
(Prepare 10 ml in deionized water using Adenosine 5'-Diphosphate, Sodium Salt, Sigma Prod. No. A-6521.)
- H. 3.0% (v/v) Hydrogen Peroxide Solution (H₂O₂)
(Prepare 10 ml in deionized water using Hydrogen Peroxide, 30% (w/w) Solution, Sigma Prod. No. H-1009.)
- I. 5.0 mM Glutamic Acid Standard Solution (Glu Std)
(Prepare 1 ml in deionized water using L-Glutamic Acid, Hydrochloride, Sigma Prod. No. G-2128. Store in an ice bath. **PREPARE FRESH.**)
- J. Glutaminase Enzyme Solution (Glutaminase)
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of Glutaminase in Reagent D.)
- K. L-Glutamic Dehydrogenase Enzyme Solution (GDH)
(Immediately before use, prepare a solution containing 70 units/ml of L-Glutamic Dehydrogenase Sigma Prod. No. G-2626 in cold deionized water.)

PROCEDURE:

Step 1:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Phos/Tris)	0.70	0.70
Reagent B (Glutamine)	0.20	0.20

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PROCEDURE: (continued)

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

Reagent J (Glutaminase)		0.10

Reagent D (Phos/Boric Acid)	-----	0.10

Immediately mix by inversion and incubate for exactly 30 minutes at 37°C. Then add:

Reagent C (HCl)	0.10	0.10
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Mix by vortexing and cool to 0-5°C in an ice bath for 5 minutes.

Step 2:

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

	<u>Test</u>	<u>Blank</u>	<u>Std</u>	<u>Std Blank</u>
Reagent E (Tris/HCl)	2.25	2.25	2.25	2.25
Reagent F (β-NAD)	0.30	0.30	0.30	0.30
Reagent G (ADP)	0.06	0.06	0.06	0.06
Reagent H (H ₂ O ₂)	0.03	0.03	0.03	0.03
Reagent K (GDH)	0.30	0.30	0.30	0.30
Reagent I (Glu Std)	-----	-----	0.06	-----
Deionized Water	-----	-----	-----	0.06

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant using a suitable spectrophotometer and record the initial A_{340nm} for the Test, Blank, Standard, and Standard Blank. Then add:

Test Solution (Step 1)	0.06	-----	-----
Blank Solution (Step 1)	-----	0.06	-----

Mix by inversion and record the increase in A_{340nm} until complete (approximately 30 minutes). Record the final A_{340nm} for the Test, Blank, Standard and Standard Blank.

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CALCULATIONS:

$$\begin{aligned} r A_{340\text{nm}} \text{ Test} &= A_{340\text{nm}} \text{ Test}_{\text{Final}} - A_{340\text{nm}} \text{ Test}_{\text{Initial}} \\ r A_{340\text{nm}} \text{ Blank} &= A_{340\text{nm}} \text{ Blank}_{\text{Final}} - A_{340\text{nm}} \text{ Blank}_{\text{Initial}} \end{aligned}$$

$$\text{Units/mg enzyme} = \frac{(A_{340\text{nm}} \text{ Test} - A_{340\text{nm}} \text{ Blank}) (1.1) (3.0)}{(6.22) (\text{mg enzyme/RM}) (0.06) (30)}$$

1.1 = Volume of assay (ml) in Step 1

3.0 = Volume of assay (ml) in Step 2

6.22 = Millimolar extinction coefficient of β -NADH at 340 nm.

RM = Reaction Mixture

0.06 = Volume of Step 1 used in Step 2

30 = Time of assay (in minutes) as per the Unit Definition

$$r A_{340\text{nm}} \text{ Standard} = A_{340\text{nm}} \text{ Standard} - A_{340\text{nm}} \text{ Standard Blank}$$

The theoretical $r A_{340\text{nm}}$ for the Standard is 0.62. It should be between 0.560 and 0.680.

UNIT DEFINITION:

One unit will deaminate 1.0 μ mole of L-glutamine per min at pH 8.6 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 184 mM potassium phosphate, 44 mM Tris, 0.18 mM ethylenediaminetetraacetic acid, 1.0 mM boric acid, 20 mM glutamine, and 0.05 - 0.10 unit glutaminase.

REFERENCE:

Curthoys, N.P. and Weiss, R.F. (1974) *Journal of Biological Chemistry* **249**, 3261-3266.

NOTES:

1. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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