SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of GLUTAMINASE[1]
(EC 3.5.1.2)
(From E. coli)

PRINCIPLE:

L-Glutamine + H_2O $\xrightarrow{\text{Glutaminase}}$ Glutamate + NH_3

CONDITIONS: T = 37°C, pH = 4.9, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 100 mM Sodium Acetate Buffer, pH 4.9 at 37°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 4.9 at 25°C with 1 M HCl.)

B. 5 mM Sodium Acetate Buffer, pH 6.0 at 37°C
(Prepare 10 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 6.0 at 25°C with 1 M HCl.)

C. 80 mM L-Glutamine Solution
(Prepare 10 ml in Reagent A using L-Glutamine, Sigma Prod. No. G-3126. **PREPARE FRESH.**)

D. Glutaminase Solution
(Immediately before use, prepare a solution containing 5 units/ml of Glutaminase in cold Reagent B.)

E. Ammonia Diagnostic Kit (171-20)
(Use Ammonia Reagent, Sigma Stock No. 171-20.)

F. Ammonia Diagnostic Kit (170-4)
(Use L-Glutamate Dehydrogenase, Sigma Stock No. 170-4)

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PROCEDURE:

Step 1:

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

	Test	Blank
Reagent A (Sodium Acetate Buffer)	0.4	0.4
Reagent C (L-Glutamine)	0.5	0.5

Equilibrate to 37°C. Then add:

Reagent D (Glutaminase)	0.1	------
Reagent B (Sodium Acetate Buffer)	------	0.1

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

Cold Deionized Water	9.0	9.0

Immediately use as Test and Blank solutions in Step 2.

Step 2:

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

	Test	Blank
Reagent E (Ammonia Reagent)	1.0	1.0

Equilibrate to 25°C. Then add:

Test Solution (10 ml)	0.1	------
Blank Solution (10 ml)	------	0.1

Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Record the initial A_{340nm} for both Test and Blank. Then add:

Reagent F (L-Glutamate Dehydrogenase)	0.01	0.01

Immediately mix by inversion and record the decrease in A_{340nm} until completion (approximately 5 minutes). Obtain the final A_{340nm} for both the Test and Blank.

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CALCULATIONS:

ΔA_{340nm} Test = A_{340nm} Test Final - A_{340nm} Test Initial

ΔA_{340nm} Blank = A_{340nm} Blank Final - A_{340nm} Blank Initial

$$\text{Units/ml enzyme} = \frac{\Delta A_{340nm}\ \text{Test} - \Delta A_{340nm}\ \text{Blank (10 ml)(1.11)}}{\text{(6.22) (15) (0.1) (0.1 ml)}}$$

6.22 = Millimolar extinction coefficient of β-NADH at 340nm
15 = Reaction time of Step 1
RM = Reaction Mixture of Step 1
10 = Final volume of Step 1
0.1 = Volume from Step 1 used in Step 2
1.11 = Step 2 Reaction Mix volume
0.1 = Volume (in milliliters) 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 deaminate 1.0 µmole of L-glutamine per minute at 37°C at pH 4.9.

FINAL ASSAY CONCENTRATION:

In a 1.0 ml reaction mix, the final concentrations are 90 mM sodium acetate, 40 mM L-glutamine and 0.5 units glutaminase.

NOTES:

1. This assay is not for the use with glutaminase from porcine kidney.

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