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Product Information

Glutamine/Glutamate Determination Kit

Catalog Number **GLN1**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The Glutamine/Glutamate Determination Kit is designed for the spectrophotometric measurement of L-glutamine and/or L-glutamate via enzymatic deamination of L-glutamine and dehydrogenation of L-glutamate with conversion of NAD^+ to NADH.

This kit may be used for the spectrophotometric measurement of L-glutamine and/or L-glutamate in liquid preparations. Pretreatment is required for the analysis of L-glutamine in serum or plasma.[1] D-isomers are not detected with this kit.

Determination of L-glutamine is a two-step reaction: (A) deamination of L-glutamine to L-glutamate and (B) dehydrogenation of the L-glutamate to α-ketoglutarate accompanied by reduction of NAD^+ to NADH. The conversion of NAD^+ to NADH is measured spectrophotometrically and is proportional to the amount of glutamate that is oxidized, hence the amount of glutamine converted to glutamate in the samples.
Note: The terms, glutamic acid and glutamate, are used interchangeably throughout this procedure.

Reaction (A): Deamination of glutamine

$$\text{L-glutamine} + H_2O \xrightarrow[H^+]{\text{glutaminase}} \text{L-glutamate} + NH_4^+$$

Reaction (B): Dehydrogenation of glutamate and reduction of NAD^+

$$\text{L-glutamate} + NAD^+ + H_2O \xrightarrow{\text{L-GLDH}} \alpha\text{-Ketoglutarate} + NH_4^+ + \text{NADH}$$

For determination of L-glutamine in medium containing both L-glutamine, and L-glutamate or its salts, endogenous L-glutamate must be measured.
See: Reaction (A); Test samples containing L-glutamine and L-glutamate.

It is recommended the entire procedure be reviewed before starting the assay.

Components

Catalog Number	Item	Quantity
A4433	0.5 M Acetate buffer, pH 5	20 ml
A4558	100 mM Adenosine 5′-Diphosphate, (ADP) Lyophilized*	1 ml
G6275	2 mM L-Glutamine, Lyophilized*	25 ml
G6150	1 mM L-Glutamic Acid,	25 ml
G5900	Glutamic Dehydrogenase (L-GLDH)	1 ml
G8880	Glutaminase 10 U/ml, Lyophilized*	5 ml
207942	Hydrazine Monohydrate (64-65% Hydrazine)	3 ml
N9268	30 mM β-Nicotinamide Adenine Dinucleotide (NAD), Lyophilized*	5 ml
T3161	Tris (0.1 M)-EDTA (0.002 M) Buffer	60 ml

* Lyophilized items should be reconstituted to the indicated volume to yield appropriate concentration, See Preparation Instructions for details.

Equipment Required but Not Provided.

- Spectrophotometer capable of reading absorbance at 340 nm.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Use ultrapure water for the preparation of components.

Preparation Of Components
1. Reconstitute L-Glutamine (Catalog Number G6275), L-Glutamate (Catalog Number G6150), NAD (Catalog Number N9268), and ADP (Catalog Number A4558) with water to volumes indicated on labels. Store reconstituted components at –20 °C in working aliquots.
2. Prepare a 1:10 dilution of Acetate Buffer (Catalog Number A4433) in water and use 5 ml of this to dissolve Glutaminase (Catalog Number G8880). Store at –20 °C after reconstitution in working aliquots.
3. Add entire contents of Hydrazine Monohydrate (Catalog Number 207942, 3 ml) to 57 ml of Tris-EDTA Buffer and adjust pH to 9.0. Smaller aliquots may be prepared using the ratio of 1 ml hydrazine to 19 ml Tris-EDTA Buffer. Keep unused hydrazine tightly closed at 2–8 °C.

Storage/Stability
The Glutamine-Glutamate Determination Kit can be stored at 2–8 °C.

Store reconstituted components at –20 °C in working aliquots.

Procedure
This is a modification of the procedure described by Lund.[1]

I. DETERMINATION OF GLUTAMINE

A. Reaction (A): Deamination Of Glutamine

Standard Curve – Use ultrapure water for the preparation of standards and samples.
1. Label appropriate size tubes STD 0.1 to 1.0. Total reaction volume of each tube should equal 1 ml.
2. Dispense components as follows:
   a. Acetate buffer (undiluted): 0.2 ml
   b. Glutaminase solution: 0.1 ml
   c. 2 mM Glutamine Standard (Gln) and water according to Table 1.

Table 1.
Preparation of Glutamine Standards

μmoles	Gln (ml)	Water (ml)
0	0*	0.7
0.1	0.05	0.65
0.2	0.1	0.6
0.3	0.15	0.55
0.4	0.2	0.5
0.5	0.25	0.45
0.6	0.3	0.4
0.7	0.35	0.35
0.8	0.4	0.3
0.9	0.45	0.25
1.0	0.5	0.2

* Tube with 0 ml of L-glutamine is the assay blank.

Test Samples without Glutamate
Note: Samples with a concentration >2 mM L-glutamine must be diluted to 2 mM or less. The amount of glutaminase used may not be sufficient to deaminate higher levels of L-glutamine.
1. Label tubes GLN. If using more than one tube, number tubes to avoid confusion.
2. Add Acetate buffer and Glutaminase as indicated for preparation of Standard Curve (See Procedure, Steps I, A, 2a and 2b).
3. Add: 0.25 ml of Test Sample and 0.45 ml of water.
   Note: If other sample volumes are used, the water volume must be adjusted to yield a total reaction volume of 1 ml.
4. Incubate all tubes at 37 °C for 1 hour.

Test Samples containing both L-glutamine and L-glutamate – For samples containing both L-glutamine and L-glutamate, endogenous L-glutamate must be determined and subtracted from L-glutamate concentration derived by deamination of L-glutamine.
1. Label and number duplicate tubes for each sample as follows: GLN+GLU for endogenous and L-glutamate derived from L-glutamine. GLU for endogenous L-glutamate.

2. Add components as indicated below for tubes marked GLN+GLU and GLU.

		GLN+GLU	GLU
a.	Acetate buffer	0.2 ml	0.2 ml
b.	Glutaminase	0.1 ml	–
c.	Test sample	0.25 ml	0.25 ml
d.	Water	0.45 ml	0.55 ml

Note: If other sample volumes are used, the water volume must be adjusted to yield a total reaction volume of 1 ml.

3. Incubate all tubes at 37 °C for 1 hour.

B. Reaction (B): Dehydrogenation of Glutamate

1. Label cuvettes: STD, GLN, GLN+GLU, or GLU as applicable.
2. Dispense components into appropriate tubes as follows:

	STD	GLN	GLN+GLU	GLU
Tris-EDTA-Hydrazine buffer	1.0 ml	1.0 ml	1.0 ml	1.0 ml
NAD solution	0.1 ml	0.1 ml	0.1 ml	0.1 ml
ADP solution	0.01 ml	0.01 ml	0.01 ml	0.01 ml
From REACTION A:				
STD tubes (including blank)	0.5 ml	–	–	–
GLN tubes	–	0.5 ml	–	–
GLN+GLU tubes	–	–	0.5 ml	–
GLU tubes	–	–	–	0.5 ml
Water	0.39 ml	0.39 ml	0.39 ml	0.39 ml

3. Place caps on cuvettes and mix by inversion.
4. Read absorbance at 340 nm to obtain background reading.
5. Add 0.02 ml of L-GLDH, mix by inversion, and hold at room temperature for 40 minutes.
6. Read absorbance at 340 nm after 40 minutes or until absorbance remains constant. Subtract background absorbance from this reading for net absorbance.
7. Glutamine Standard Curve: Graph the L-glutamine standards using μmoles vs. absorbance.
   Note: Unknown concentrations for L-glutamine and L-glutamate can be determined from the graph.
8. Calculation of L-glutamine in test sample: To determine concentration of L-glutamine in sample, divide the observed result by 0.25 ml or the volume of sample that was used in the test. Multiply the result by the reciprocal of any dilutions that were made.
   Note: μmoles/ml = mmoles/L
9. For samples containing both L-glutamine and L-glutamate, subtract endogenous L-glutamate concentration from total L-glutamate concentration. The result is the concentration of L-glutamate arising from deamination of L-glutamine.

II. DETERMINATION OF GLUTAMATE

Note: Reaction (B) may be used without Reaction (A) to determine L-glutamate or its salts without determining L-glutamine.

A. Construct a glutamate standard curve as shown below to determine L-glutamate in test samples.

1. Label cuvettes S (standard) or T (test samples).
2. Dispense components as follows:

Tris-EDTA-Hydrazine buffer	1.0 ml
NAD solution	0.1 ml
ADP solution	0.01 ml
Glutamate standards or samples	(See Table 2)
Water	(See Table 2)

Table 2.
Preparation of Glutamate Standards (Glu)

μmoles	Glu (ml)	Water (ml)
0	0*	0.89
0.05	0.05	0.84
0.1	0.1	0.79
0.15	0.15	0.74
0.2	0.2	0.69
0.25	0.25	0.64
0.3	0.3	0.59
0.35	0.35	0.54
0.4	0.4	0.49
0.45	0.45	0.44
0.5	0.5	0.39

* Tube with 0 ml of L-glutamate is the assay blank.

B. Test samples:

1. Add 0.25 ml of test samples in place of the glutamate standard and 640 μl of water. If other sample volumes are used, the water volume must be adjusted to yield a total reaction volume of 2 ml.
   Note: Samples with L-glutamate >1 mM should be diluted to 1 mM or less to fall within the standard curve.
2. Place caps on cuvettes and mix by inversion.
3. Read absorbance at 340 nm to obtain background reading.

4. Add 0.02 ml of L-GLDH, mix by inversion, and hold at room temperature.
5. Read absorbance at 340 nm after 40 minutes until absorbance remains constant. Subtract background from this for net absorbance.
6. Glutamate Standard Curve: Graph L-glutamate standards using mM versus absorbance and determine unknown values from this graph.
7. Calculation of L-glutamate in test sample: To determine concentration of L-glutamate in sample, divide the observed results by 0.25 ml or the volume of sample that was used in the reaction. Multiply this by the reciprocal of any dilutions that were made.
   Note: μmoles/ml = mmoles/L

Reference

1. Lund, P., L-Glutamine and L-Glutamate: UV-Method with Glutaminase and Glutamate Dehydrogenase. In Methods of Enzymatic Analysis, Volume 8, H.U. Bergmeyer, (ed). VCH, Verlagsgesellschaft, (Weinheim, 1986) pp 357-363.

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