Product Information

Glutamine Colorimetric Assay Kit

I. Kit Contents:

Components	K2084-100 100 assays	Cap Color	Part Number
Hydrolysis Buffer	25 ml	NM	K2084-C-1
Development Buffer Hydrolysis Enzyme	25 ml	WM	K2084-C-2
Mix (Lyophilized) Development Enzyme	1 vial	Blue	K2084-C-3
Mix (Lyophilized)	1 vial	Green	K2084-C-4
Developer (Lyophilized)	1 vial	Red	K2084-C-5
Gln Standard (Lyophilized)	1 vial	Yellow	K2084-C-6

II. Introduction:

Glutamine (Gln) is a non-essential amino acid and is synthesized via condensation of glutamate and ammonia. Gln is the most abundant free amino acid in human blood. Gln plays critical roles in protein synthesis, cell growth, and regulation of acid-balance in mammalian kidneys. Gln is also the main source of nitrogen for the synthesis of hexosamines and nucleotides. Glutamine is benefit for patients suffering from severe burns, Crohn's disease, cancer and HIV/AIDS.

The Glutamine Colorimetric Assay Kit provides a sensitive and simple way for detection of glutamine in various biological samples based on colorimetric method. The assay is based on the hydrolysis of Glutamine to Glutamate producing a stable signal, which is directly proportional to the amount of Gln in the sample. The assay can detect as little as 25 µM of Gln in various biological samples.

III. Application:

Measurement of Glutamine in various biological samples.

IV. Sample Type:

Serum, plasma, urine or other biological fluids.

Mammalian tissues: kidney, liver, brain samples, etc.

V. User Supplied Reagents and Equipment:

96-well clear plate with flat bottom.

Multi-well spectrophotometer.

10K Spin Column.

VI. Storage Conditions and Reagent Preparation:

Store kit at -20ÁC protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

Hydrolysis Buffer and Development Buffer: Bring to room temperature before use. Store at -20ÁC Stable for two months.

Hydrolysis Enzyme Mix: Reconstitute with 22 µl Hydrolysis Buffer to make the stock solution. Pipette gently to dissolve. Store at -20ÁC Keep on ice while in use. Stable for two months.

Development Enzyme Mix: Reconstitute with 220 µl Development Buffer. Pipette gently to dissolve. Aliquot & store at -20ÁC Keep on ice while in use. Stable for two months.

Developer: Reconstitute with 220 µl Development Buffer. Pipette gently to dissolve. Aliquot & store at -20ÁC Keep on ice while in use. Stable for two months.

Gln Standard: Reconstitute with 100 µl ddH_2O to generate 10 mM solution. Store at -20ÁC Stable for two months.

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VII. Glutamine Assay Protocol:

1. Sample Preparation: Centrifuge biological fluids at 10,000 X g for 5 min. at 4ÁC Collect the supernatant & add 1-40 µl into desired well(s) in a 96-well plate. For mammalian tissues, homogenize ~10-20 mg of tissue on ice using 10x (v/w) Hydrolysis Buffer. Centrifuge the homogenate at 10,000 X g, 10 min. at 4ÁC Collect the supernatant & add 1 - 40 µl into desired well(s) in a 96-well plate. Adjust the volume to 40 µl/well with ddH_2O.

Notes:

a. Glutamine concentrations can vary over a wide range depending on the sample. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

b. Glutamate in the sample will contribute to the background signal. If high glutamate levels are predicted in the sample, prepare parallel sample well(s) as sample background control(s).

c. For samples having high protein content, we recommend deproteinizing the samples (tissue lysate or biological fluids) using 10K Spin Column. Add sample to the spin column, centrifuge at 10,000 X g for 10 min. at 4°C Collect the filtrate.

d. Endogenous compounds may interfere with the assay. To ensure accurate determination of Gln in the test samples or for samples having low concentration of Gln, we recommend spiking samples with a known amount of Gln Standard (6 nmol).

2. Standard Curve Preparation: Dilute Gln Standard to 1 mM by adding 10 µl of 10 mM Gln Standard to 90 µl of ddH_2O. Add 0, 2, 4, 6, 8 and 10 µl of Gln Standard into series of wells in a 96-well plate to generate 0, 2, 4, 6, 8 & 10 nmol/well of Gln Standard. Adjust the volume to 40 µl/well with ddH_2O.

3. Hydrolysis Mix: Dilute Hydrolysis Enzyme Mix stock solution 10 times by adding 1 µl of stock solution into 9 µl of Hydrolysis Buffer as needed. Mix enough reagents for the number of assays to be performed. For each well, prepare 10 µl of Hydrolysis Mix.

	Hydrolysis Mix	Background Hydrolysis Mix
Hydrolysis Buffer	9 µl	10 µl
Diluted Hydrolysis Enzyme Mix	1 µl	---

Mix & add 10 µl of Hydrolysis Mix to Standard & sample wells. Adjust the volume to 50 µl/well with ddH2O. Incubate for 30 min. at 37°C

For samples having high glutamate levels, add 10 µl of Background Hydrolysis Mix to sample background control well(s). Adjust the volume to 50 µl/well with ddH_2O & incubate for 30 min. at 37°C.

Note: Always prepare fresh diluted Hydrolysis Enzyme Mix. Don't store the diluted Hydrolysis Enzyme Mix.

4. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix
Development Buffer	46 µl
Development Enzyme Mix	2 µl
Developer	2 µl

Mix well. Add 50 µl of the Reaction Mix to each well containing Standards, samples and Background Control(s). Mix well.

5. Measurement: Incubate at 37°C for 60 min., protected from light. Measure absorbance (OD 450 nm) in a plate reader.

6. Calculation: Subtract 0 Gln Standard reading from all readings. Plot the Gln Standard Curve. If sample Background Control reading is significant, then subtract sample Background Control reading from sample reading. Apply the corrected OD to the Gln Standard Curve to get B nmol of Gln in the sample well.

Sample Gln concentration (C) = B/V × D nmol/µl or mM

Where: B is the amount of Gln in the sample well from Standard Curve (nmol).

V is the sample volume added into the reaction well (µl).

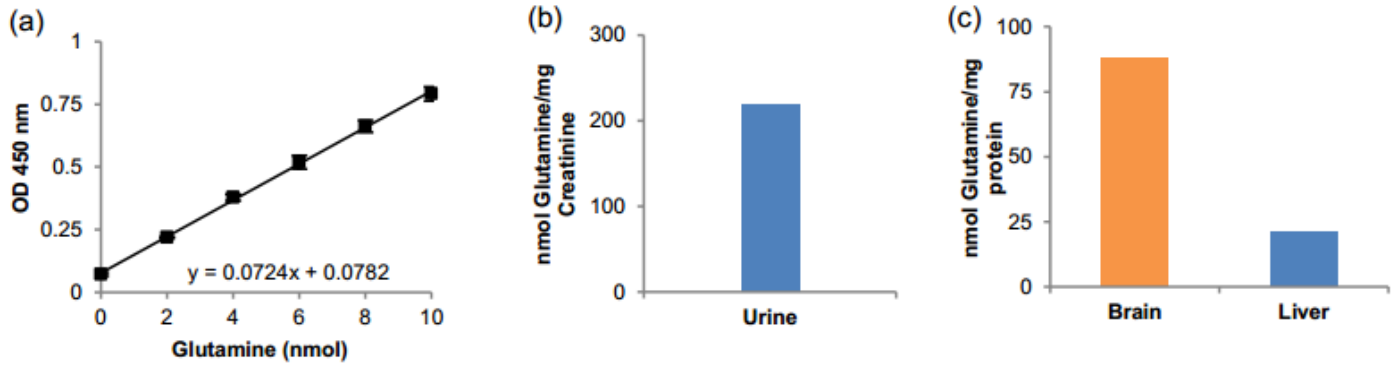
D is the sample dilution factor.

Note: For spiked samples, correct for any sample interference by using following equation:

For spiked samples, Gln amount in sample well (B) = (OD sample (corrected))/ [sample + Gln Std(corrected)× OD sample (corrected))]* Gln Std(corrected) (nmol)

Gln concentration can also be expressed as nmol/mg of protein or nmol/mg of creatinine in case of urine.

Glutamine molecular weight: 146.1 g/mol.



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Our promise

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