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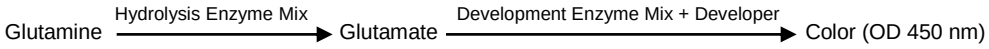
Glutamine Colorimetric Assay Kit

10/16

(Catalog # K556-100; 100 assays; Store at -20°C)

I. Introduction:

Glutamine (Gln) is one of the most abundant amino acids containing an uncharged amide as a side chain. It is synthesized via condensation of glutamate and ammonia. Gln is classified as a non-essential amino acid, however, Gln is important in several biological processes such as protein synthesis, regulation of acid-balance in mammalian kidneys and cell growth. It constitutes cell's main source of nitrogen for the synthesis of nucleotides and hexosamines. Glutamine-rich diets benefit patients suffering from Crohn's disease, severe burns, HIV/AIDS and cancer. BioVision's Glutamine Colorimetric Assay kit is a simple and sensitive assay that detects the biologically relevant concentrations of Gln in various fluids and tissues. 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 a variety of biological samples.



II. Application:

- Measurement of Glutamine in various biological samples

III. Sample Type:

- Serum, plasma, urine or other biological fluids
- Mammalian tissues: kidney, liver, brain samples etc.

IV. Kit Contents:

Components	K556-100	Cap Code	Part Number
Hydrolysis Buffer	25 ml	NM	K556-100-1
Development Buffer	25 ml	WM	K556-100-2
Hydrolysis Enzyme Mix (Lyophilized)	1 vial	Blue	K556-100-3
Development Enzyme Mix (Lyophilized)	1 vial	Green	K556-100-4
Developer (Lyophilized)	1 vial	Red	K556-100-5
Gln Standard (Lyophilized)	1 vial	Yellow	K556-100-6

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- 10K Spin Column (Cat # 1997)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, 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 -20°C. Stable for two months.
- **Hydrolysis Enzyme Mix:** Reconstitute with **220 µl Hydrolysis Buffe**r to make the stock solution. Pipette gently to dissolve. Store at -20°C. 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 -20°C. Keep on ice while in use. Stable for two months.
- **Developer:** Reconstitute with **220 µl Development Buffer**. Pipette gently to dissolve. Aliquot & store at -20°C. 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 -20°C. Stable for two months.

VII. Glutamine Assay Protocol:

1. **Sample Preparation:** Centrifuge biological fluids at 10,000 X g for 5 min. at 4°C. 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 4°C. 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 (Cat # 1997). 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:** Add 2 µl Hydrolysis Enzyme mix to the Standard and Sample wells as follows:

	Standard/Sample	*Sample Background
Hydrolysis Enzyme Mix	2 µl	---
Hydrolysis Buffer	8 µl	10 µl

Mix well. Adjust the volume to 50 µl/well with ddH_2O if necessary. Incubate for 30 min. at 37 °C.

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

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 X 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:

$$\textbf{Gln amount in spiked sample well (B)} = \left(\frac{(\mathrm{OD}_{\text{sample (corrected)}})}{(\mathrm{OD}_{\text{sample + Gln Std(corrected)}})\text{-}(\mathrm{OD}_{\text{sample(corrected)}})}\right) * Gln\ Spike\ (\mathrm{nmol})$$

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

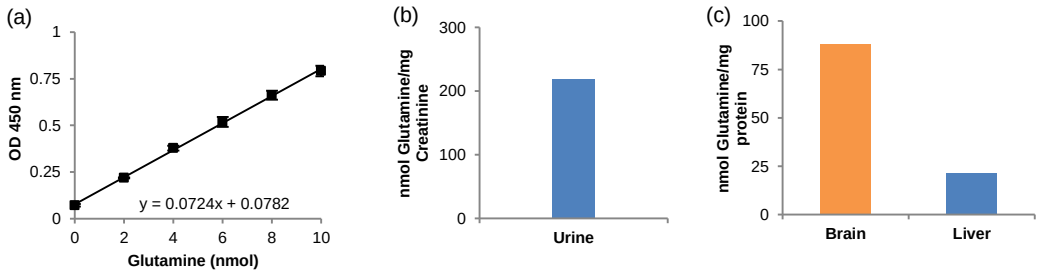


Figure: Glutamine Standard Curve (a). Measurement of Glutamine concentration in human urine (5 µl) (b) & mouse tissues (brain [15 µg], liver [50 µg]) (c). Samples were deproteinized using 10K Spin Column (Cat # 1997) & spiked with known amount of Glutamine (6 nmol). Assay was performed following the kit protocol.

VIII. Related Products:

Phenylalanine Fluorometric Assay Kit (K572)
Aspartate Colorimetric/Fluorometric Assay Kit (K552)
Branched Chain Amino Acid (Leu/Ile/Val) Colorimetric Assay Kit (K564)
Tyrosine Colorimetric Assay Kit (K573)
Asparaginase Activity Colorimetric/Fluorometric Assay Kit (K754)
L-Amino Acid Quantitation Colorimetric/Fluorometric Assay Kit (K639)
10K Spin Column (1997)
Glutamate Colorimetric Assay Kit (K629)
Alanine Colorimetric/Fluorometric Assay Kit (K652)
Hydroxyproline Colorimetric Assay Kit (K555)
Arginase Activity Colorimetric Assay Kit (K755)
Urea Colorimetric Assay Kit (K375)
Urea Colorimetric Assay Kit II (K376)

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