Glutamine Fluorometric/Colorimetric Assay Kit

Item No. 702430

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
400611	Glutamine Assay Buffer 1 (5X)	1 vial/8 ml	RT
400612	Glutamine Assay Buffer 2	1 vial/8 ml	RT
400610	MaxiProbe	1 vial/250 µl	-20°C
400613	Glutamine Assay Standard	1 vial/100 µl	-20°C
400614	Glutamine Converter Enzyme	1 vial	-20°C
400615	Glutamine/Glutamate Developer Enzyme Mix	1 vial	-20°C
400115	Half-Area 96-Well Solid Plate (black, clear bottom)	1 plate	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Glutamine Fluorometric/Colorimetric Assay Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring fluorescence with excitation and emission wavelengths of 535±5 and 590±10 nm, respectively, or a plate reader capable of measuring absorbance at 570 nm
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of pure water; glass-distilled water is acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Anhydrous DMSO (cell culture grade is preferred)
5. Aluminum foil
6. Materials used for **Sample Preparation** (see page 10)
7. A 37°C incubator

INTRODUCTION

Background

Glutamine is a conditionally essential amino acid and metabolite involved in diverse biochemical processes.[1,2] It is a proteinogenic amino acid and contributes to purine, pyrimidine, and hexosamine biosynthesis. Glutamine is primarily biosynthesized by skeletal muscle *via* amination of glutamate by glutamine synthetase but is also obtained in the diet.[1,3] In the kidney, it acts as a donor of ammonia, which participates in pH regulation of the blood.[1] In the CNS, it is a source of the excitatory neurotransmitter glutamate and, subsequently, the inhibitory neurotransmitter GABA.[4] Glutamine degradation (glutaminolysis) is upregulated in cancer, and decreased serum levels of glutamine are associated with poor prognosis in patients with colorectal cancer.[5,6] It is released from skeletal muscle in response to injury, and plasma levels of glutamine are decreased in overly trained athletes and after periods of prolonged exercise.[7,8] Glutamine has commonly been used in cell culture media as a carbon and nitrogen source.[1]

About This Assay

Cayman's Glutamine Fluorometric/Colorimetric Assay Kit provides both fluorometric and colorimetric methods for measuring glutamine in biological samples such as cell culture supernatants, cell lysates, tissue homogenates, serum, and plasma. In the assay, the Glutamine Converter Enzyme catalyzes the conversion of glutamine to glutamate, which is further converted to α-ketoglutarate (α-KG) and hydrogen peroxide (H_2O_2) in the presence of the Glutamine/Glutamate Developer Enzyme Mix. MaxiProbe reacts with H_2O_2, forming a compound that can be easily quantified at excitation and emission wavelengths of 535±5 and 590±10 nm, respectively (Figure 1). Alternatively, the absorbance of the product can be measured at 570 nm. Background from glutamate and H_2O_2 in the sample is determined by omitting the converter enzyme in the sample background reactions.

This assay offers the option of measuring fluorescence or absorbance. It is at the user's discretion to choose the mode of measurement (and corresponding standard curve preparation) that best fits their needs. When read fluorometrically, this assay has a range of 0-40 µM and a lower limit of detection (LLOD) of 1.6 µM. When read colorimetrically, the range is 0-400 µM with an LLOD of 2.3 µM.

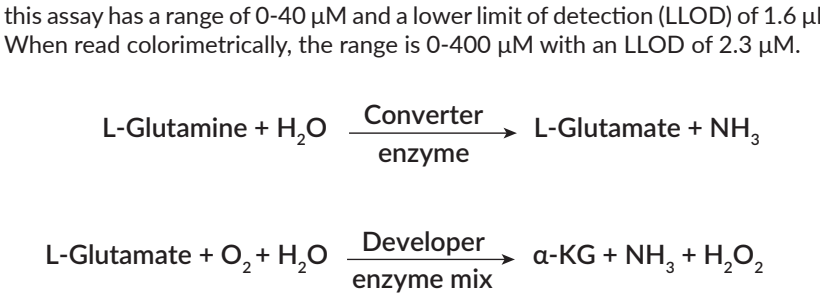


Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

NOTE: It is normal for the concentrated buffers to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.

1. **Glutamine Assay Buffer 1 (5X) - (Item No. 400611)**

   This vial contains 8 ml of Glutamine Assay Buffer 1 (5X). Thaw at room temperature and dilute the entire contents of the vial with 32 ml of pure water. Be certain to rinse the vial to remove any salts that may have precipitated. Glutamine Assay Buffer 1 (1X) will be stable for at least two months when stored at room temperature.

2. **Glutamine Assay Buffer 2 - (Item No. 400612)**

   This vial contains 8 ml of Glutamine Assay Buffer 2. Thaw at room temperature. It is ready to use as supplied.

3. **MaxiProbe - (Item No. 400610)**

   This vial contains 250 µl of MaxiProbe. Add 90 µl of DMSO to the vial and mix well. This is a sufficient volume to evaluate 100 wells. The MaxiProbe solution will be stable for one hour at room temperature if capped and protected from light. If not using the diluted MaxiProbe all at once, prepare aliquots and store at -20 °C. Avoid more than three freeze-thaw cycles.

4. **Glutamine Assay Standard - (Item No. 400613)**

   This vial contains 100 µl of 4 mM glutamine. If all of the Glutamine Assay Standard will not be used at once, aliquot and store at -20°C. Avoid more than three freeze-thaw cycles.

   Prior to each assay, add 20 µl of Glutamine Assay Standard to 180 µl of Glutamine Assay Buffer 1 (1X) to make a 400 µM bulk standard. Use this bulk standard in the preparation of the standard curves as shown on pages 14 and 15.

5. **Glutamine Converter Enzyme - (Item No. 400614)**

   This vial contains lyophilized Glutamine Converter Enzyme. Reconstitute the contents of the vial with 300 µl of pure water and place on ice. Do not vortex. The reconstituted Glutamine Converter Enzyme will be stable for four hours on ice. If not using the reconstituted enzyme all at once, prepare aliquots, store at -20°C, and use within two months. Avoid more than three freeze-thaw cycles.

   Prior to use in the assay, dilute 100 µl of the reconstituted enzyme with 4,900 µl of Glutamine Assay Buffer 1 (1X). This is a sufficient volume to assay 100 wells. Scale down as needed. The diluted Glutamine Converter Enzyme will be stable for four hours on ice.

6. **Glutamine/Glutamate Developer Enzyme Mix - (Item No. 400615)**

   This vial contains lyophilized Glutamine/Glutamate Developer Enzyme Mix. Reconstitute the contents of the vial with 300 µl of pure water and store on ice. Do not vortex. One vial provides a sufficient volume to assay 100 wells. The reconstituted developer enzyme mix will be stable for four hours on ice. If not using the reconstituted enzyme mix all at once, prepare aliquots, store at -80°C, and use within two months. Avoid more than three freeze-thaw cycles.

Sample Preparation

Plasma

Collect blood in vacutainers containing heparin, EDTA, or sodium citrate. To obtain plasma, centrifuge blood at 1,000 x g for 15 minutes at 4°C. Transfer the top plasma layer into a clean test tube without disturbing the white buffy layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C. It is recommended that plasma be deproteinated using ultrafiltration with 10 kDa spin filters, following the manufacturer's protocol. To fall within the range of the standard curves, it may be necessary to dilute samples with Glutamine Assay Buffer 1 (1X) prior to the assay. Glutamine levels in plasma typically range between 200 and 700 µM.

Serum

Collect blood in vacutainers without an anticoagulant. Allow samples to clot undisturbed for 30-60 minutes at room temperature. To obtain serum, centrifuge at 1,000-2,000 x g for 15-30 minutes at 4°C. Transfer the top serum layer into a clean test tube. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C. It is recommended that serum be deproteinated using ultrafiltration with 10 kDa spin filters, following the manufacturer's protocol. To fall within the range of the standard curves, it may be necessary to dilute samples with Glutamine Assay Buffer 1 (1X) prior to the assay. Glutamine levels in serum typically range between 200 and 700 µM.

Tissue Homogenate

Tissue should be processed immediately or stored at -80°C.

1. Collect tissue and mince into small pieces.
2. Prepare a homogenization buffer by combining 4 ml of 50 mM Tris HCl, pH 7.5, with 0.5 ml of 0.6 M HCl. This is a sufficient volume to process 100 mg of tissue.
3. Per 100 mg of minced tissue, add 4.5 ml of homogenization buffer and homogenize using a tissue tearor (or similar instrument) for 20-30 seconds.
4. Neutralize with 500 µl of 0.6 M Tris, pH 8.5, then centrifuge at 3,700 x g for 20 minutes at 4°C.
5. Transfer the supernatant to another tube and store on ice or at -80°C for long-term storage.

NOTE: It is recommended that samples be deproteinated prior to the assay using ultrafiltration with 10 kDa spin filters, following the manufacturer's protocol. Glutamine levels in tissue homogenates can vary widely depending on the tissue type, as well as the method of sample preparation. To fall within the range of the standard curves, it may be necessary to dilute samples with Glutamine Assay Buffer 1 (1X) prior to the assay.

Cell Lysate

1. For adherent cells grown in a 96-well plate, gently rinse the cells along with no-cell controls twice with 200 µl of pre-warmed PBS (37°C). Gently aspirate the PBS from all wells.
2. Prepare a lysis buffer by combining 8 ml of PBS with 2 ml of 0.6 M HCl.
3. Add 45 µl of lysis buffer to each well. Shake the plate for at least 10 minutes at room temperature.
4. Neutralize by adding 9 µl of 0.6 M Tris, pH 8.5, to each well, and gently mix. Generally, the lysate does not require further dilution. The fluorometric assay is recommended for cell lysates.

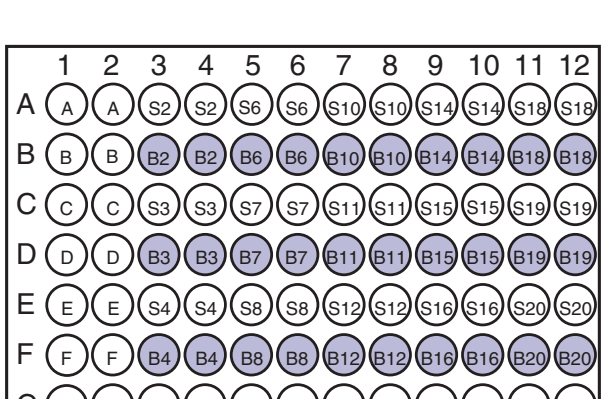
Cell Culture Supernatant

Centrifuge cells at 1,200 x g for 10 minutes at 4°C and collect the supernatant. Dilute the supernatant with Glutamine Assay Buffer 1 (1X) for use in the assay. The recommended minimum dilutions are 1:100 and 1:10 for the fluorometric and colorimetric methods, respectively. Additional dilutions may be necessary for certain samples to fall within the range of the standard curve. Phenol red does not interfere with the assay if samples are diluted as recommended.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. It is suggested that each sample and standard be assayed at least in duplicate. A typical layout of standard, sample, and sample background wells to be measured in duplicate is shown in Figure 2. It is suggested that the contents of each well be recorded on the template sheet provided (see page 29).



A-F = Standards
S1-S21 = Sample Wells
B1-B21 = Sample Background Wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume in the assay is 100 µl in all of the wells.
- All reagents should be prepared as described above. The reconstituted Glutamine Converter Enzyme and Glutamine/Glutamate Developer Enzyme Mix should be kept on ice and all other reagents should be kept at room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples be assayed at least in duplicate, but it is at the user's discretion to do so.
- The assay is performed at 37°C.
- Monitor the fluorescence with excitation and emission wavelengths of 535±5 and 590±10 nm, respectively, or monitor the absorbance at 570 nm.

Standard Curve Preparation

NOTE: This assay can be read using fluorescence or absorbance. Choose the standard curve preparation that matches the format needed. Both standard curves do not need to be prepared.

Fluorometric Standard Curve Preparation

Use the 400 µM bulk standard (See **Reagent Preparation**, page 8) to prepare the fluorometric assay standards.

Label six clean test tubes A-F. Pipette 180 µl of Glutamine Assay Buffer 1 (1X) to tube A. Pipette 100 µl of Glutamine Assay Buffer 1 (1X) to tubes B-F. Transfer 20 µl of the bulk standard (400 µM) to tube A. Mix gently. Serially dilute the standard by removing 100 µl from tube A and placing it into tube B. Mix gently. Next, remove 100 µl from tube B and place it into tube C. Mix gently. Repeat the process for tubes D-E. Do not add any standard to tube F.

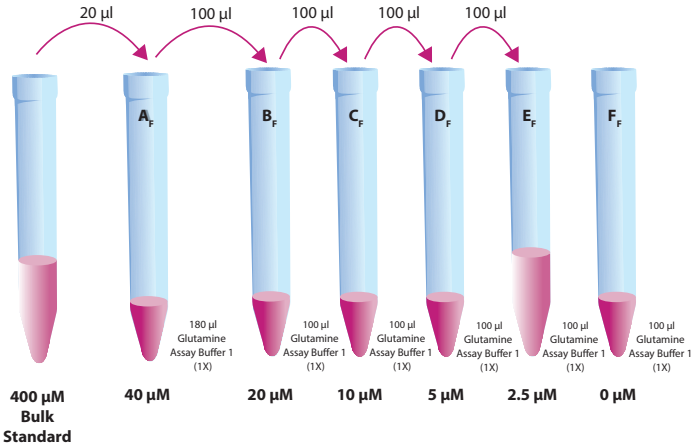


Figure 3. Preparation of the glutamine standards for the fluorometric assay format

Colorimetric Standard Curve Preparation

Use the 400 µM bulk standard (See **Reagent Preparation**, page 8) to prepare the colorimetric assay standards.

Label the bulk standard tube A. It will be the first standard. Label five clean test tubes B-F. Pipette 100 µl of Glutamine Assay Buffer 1 (1X) to tubes B-F. Serially dilute the standard by removing 100 µl from tube A and placing it into tube B. Mix gently. Next, remove 100 µl from tube B and place it into tube C. Mix gently. Repeat the process for tubes D-E. Do not add any standard to tube F.

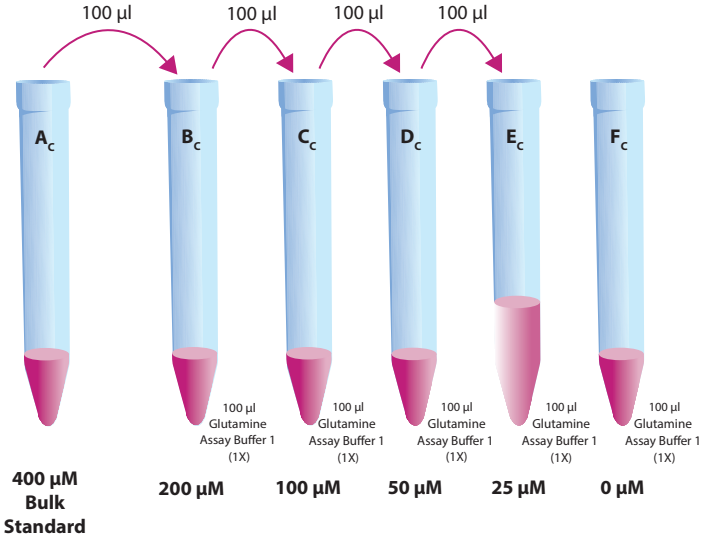


Figure 4. Preparation of the glutamine standards for the colorimetric assay format

Performing the Assay

1. Add reagents to the designated wells as follows:

Reagent	Standard	Sample	Sample Background
Diluted Glutamine Converter Enzyme	40 µl	40 µl	--
Glutamine Assay Buffer 1 (1X)	--	--	40 µl
Sample	--	10 µl	10 µl
Standard	10 µl	--	--

Table 1. Pipetting summary

2. Mix wells gently.
3. Cover the plate with aluminum foil and incubate for 15 minutes at 37°C.
4. **Prepare Development Mix immediately prior to use:** Combine Glutamine Assay Buffer 2, reconstituted Glutamine/Glutamate Developer Enzyme Mix, and diluted MaxiProbe according to the table. Scale as needed. Use the Development Mix within 15 minutes of preparation.

Reagent	50 Wells	100 Wells
Glutamine Assay Buffer 2	3.84 ml	5.76 ml
Reconstituted Glutamine/Glutamate Developer Enzyme Mix	80 µl	120 µl
Diluted MaxiProbe	80 µl	120 µl
Total Volume:	4 ml	6 ml

Table 2. Development Mix preparation

5. Add 50 µl of Development Mix to all wells being used. Mix gently by pipetting.
6. Cover with aluminum foil and incubate for 30 minutes at 37°C.
7. Remove the aluminum foil and read fluorescence using excitation and emission wavelengths of 535±5 and 590±10 nm, respectively. If the colorimetric method is used, read absorbance at 570 nm.

ANALYSIS

Calculations

1. Determine the average fluorescence or absorbance of each standard, sample, and sample background well.
2. Subtract the fluorescence or absorbance of standard F from itself and all other standards, samples, and sample background wells to obtain corrected signal values.
3. Plot the corrected signal values of each standard as a function of the concentration of glutamine. See Figures 5 and 6, on pages 20 and 21, for typical standard curves.
4. Subtract the average corrected signal values of the sample background wells from the average corrected signal values of the sample wells to yield the corrected sample measurement (CSM).
5. Calculate the glutamine concentration of the samples using the equation obtained from the linear regression of the standard curve substituting the CSM for each sample.

$$[\text{glutamine}]\ (\mu M) = \left[\frac{\text{CSM - (y-intercept)}}{\text{slope}} \right] \text{x sample dilution factor}$$

Performance Characteristics

The assay range is 0-40 µM when read fluorometrically and 0-400 µM when read colorimetrically.

The LLOD is defined as the glutamine concentration two standard deviations above the mean zero value. The LLOD for the fluorometric assay is 1.6 µM, and the LLOD for the colorimetric assay is 2.3 µM.

The lower limit of quantification (LLOQ) is the lowest standard concentration in which absorbance (450 nm) – (1.64 x S.D.) is higher than the mean zero value of absorbance (450 nm) + (1.64 x S.D.). The LLOQ for the fluorometric assay is 2.5 µM, and the LLOQ for the colorimetric assay is 25 µM.

Precision

When a series of 24 human EDTA plasma measurements were performed on the same day, the intra-assay coefficients of variation were 1.6 and 3.3% in the fluorometric and colorimetric assays, respectively. When a series of six human EDTA plasma and serum measurements were performed on three different days under the same experimental conditions, the inter-assay coefficients of variation were 14 and 17%, respectively, in the fluorometric format and 7 and 14%, respectively, in the colorimetric format.

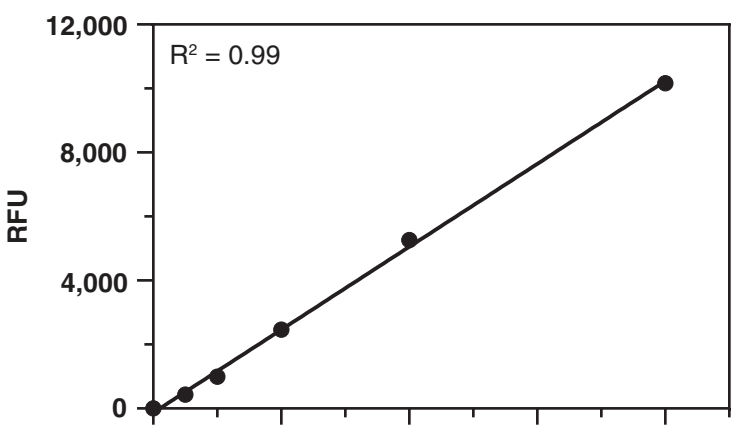


Figure 5. Glutamine standard curve for the fluorometric format

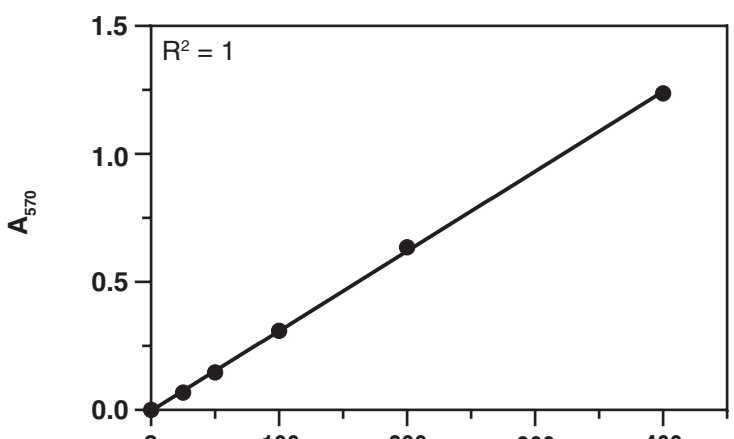


Figure 6. Glutamine standard curve for the colorimetric format

Parallelism

To assess parallelism, samples were serially diluted with Glutamine Assay Buffer 1 (1X), and evaluated using the Glutamine Fluorometric/Colorimetric Assay Kit. Measured concentrations were plotted as a function of the sample dilution. The results are shown below.

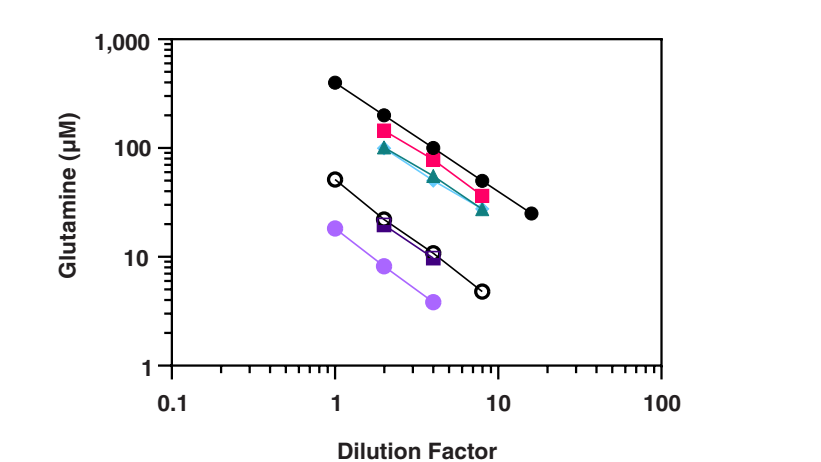


Figure 7. Parallelism of various matrices in the Glutamine Fluorometric/ Colorimetric Assay Kit

Spike and Recovery

Human EDTA plasma was spiked with glutamine, serially diluted with Glutamine Assay Buffer 1 (1X), and evaluated using the Glutamine Fluorometric/Colorimetric Assay Kit. The results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

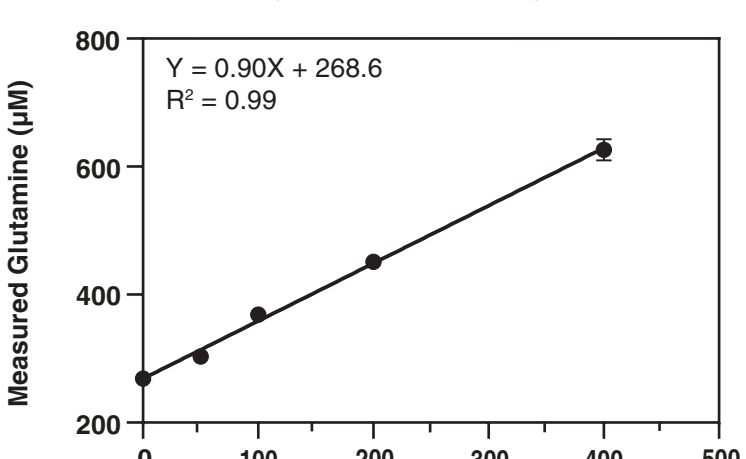


Figure 8. Spike and recovery of glutamine in human EDTA plasma using the fluorometric format

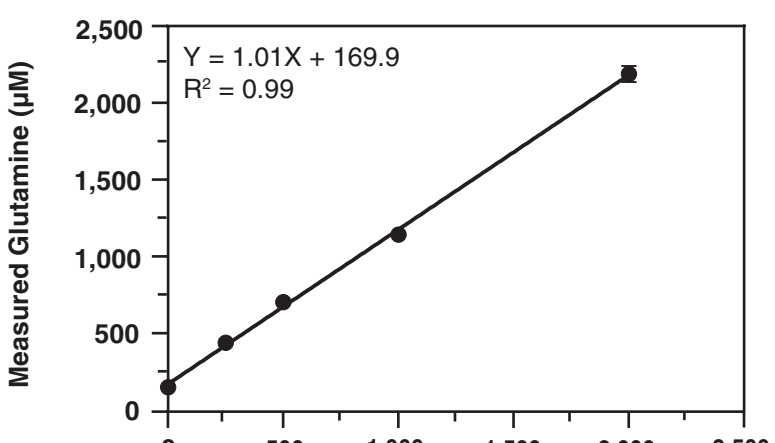


Figure 9. Spike and recovery of glutamine in human EDTA plasma using the colorimetric format

Interferences

T-PER, M-PER and RIPA buffers will interfere with this assay. If a buffer not specified in the Sample Preparation must be used to prepare samples, it is recommended that standards are prepared using the same buffer to account for any possible interferences.

Troubleshooting

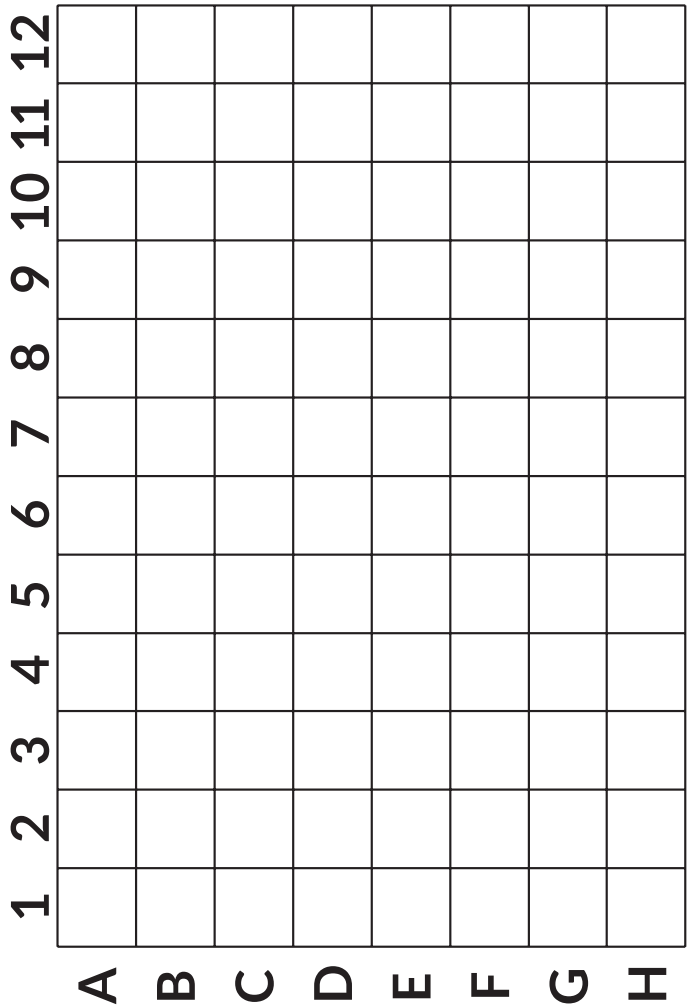
Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s) C. The reaction mix was not sufficiently mixed	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No fluorescence or absorbance was detected above standard F in the sample wells	A. The glutamine concentration is too low to detect or sample does not contain glutamine B. The sample type contains interfering substances	A. Re-assay the sample using a lower dilution or extract more sample using less buffer B. Perform serial dilutions of sample using Glutamine Assay Buffer 1 (1X) to determine if glutamine is detectable at a higher dilution
Glutamine concentration was above the highest point in the standard curve	The glutamine concentration was too high in the sample	Dilute samples with Glutamine Assay Buffer 1 (1X) and re-assay *NOTE: Remember to account for the dilution factor when calculating the glutamine concentration*
The fluorometer exhibited 'MAX' value for the wells	The *gain* setting is too high	Reduce the *gain* and re-read

Item No.	Reagent	Procedure
400611	Glutamine Assay Buffer 1 (5X)	Dilute to 1X with pure water
400612	Glutamine Assay Buffer 2	Ready to use as supplied
400610	MaxiProbe	Add 90 µl of DMSO to the vial and mix
400613	Glutamine Assay Standard	Dilute 1:10 with Glutamine Assay Buffer 1 (1X) prior to preparing standards (see Figures 3 and 4 on pages 14 and 15, respectively)
400614	Glutamine Converter Enzyme	Reconstitute with 300 µl of pure water; dilute 1:50 with Glutamine Assay Buffer 1 (1X) prior to use
400615	Glutamine/Glutamate Developer Enzyme Mix	Reconstitute with 300 µl of pure water

Table 3. Reagent preparation summary

	Standard Wells	Sample Wells	Sample Background Wells
Add Diluted Glutamine Converter Enzyme	40 µl	40 µl	--
Add Glutamine Assay Buffer 1 (1X)	--	--	40 µl
Add Standards	10 µl	--	--
Add Sample	--	10 µl	10 µl
Gently mix, cover, and incubate for 15 minutes at 37°C			
Prepare Development Mix: Mix Glutamine Assay Buffer 2, reconstituted Glutamine/Glutamate Developer Enzyme Mix, and diluted MaxiProbe at a 48:1:1 ratio			
Add Development Mix	50 µl	50 µl	50 µl
Gently mix, cover, and incubate for 30 minutes 37°C			
Read: Ex = 535±5 nm; Em = 590±10 nm OR A_{570}			

Table 4. Assay summary



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NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.