Glutaminase Activity Assay Kit

Item No. 702690

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
400666	Glutaminase Assay Buffer (5X)	1 vial/10 ml	4°C
400667	Glutaminase Substrate	1 vial/500 µl	-20°C
400668	Glutaminase Positive Control	1 vial	-20°C
400552	Glutamate Standard	1 vial/50 µl	-20°C
400615	Glutamine/Glutamate Developer Enzyme Mix	1 vial	-20°C
400610	MaxiProbe	1 vial/250 µl	-20°C
700020	Half-Volume 96-Well Clear Plate	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.

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 absorbance at 570 nm
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of pure water; glass-distilled water or HPLC-grade water is acceptable. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*
4. Non-adhesive foil
5. Materials used for **Sample Preparation** (see page 10)

INTRODUCTION

Background

Glutaminase is an amidohydrolase that catalyzes the catabolism of the amino acid glutamine to glutamate and ammonia.[1] *GLS* encodes glutaminase 1, also known as kidney-type (K-type) glutaminase, and *GLS2* encodes glutaminase 2, also known as liver-type (L-type) glutaminase.[2] Each gene product has two isoforms formed by alternative transcription. Glutaminase 1 isoforms are widely expressed in non-hepatic tissues and have a high affinity for glutamine while glutaminase 2 isoforms are expressed primarily in the liver, but also in the brain, pancreas, and immune cells.[1-3] Glutaminase is crucial for glutamine/glutamate homeostasis in the brain as it is the primary producer of glutamate, the major excitatory neurotransmitter in the mammalian brain.[2] Glutaminase 1 is pro-oncogenic and is overexpressed in a variety of cancer types.[3] The role of glutaminase 2 in cancer is more complex. It is also overexpressed in a variety of cancers, including *MYCN*-amplified neuroblastoma cells, where it is associated with poor survival, but its expression in tumors is lower than non-cancerous tissues in other cancers, such as glioblastoma, where its expression is negatively associated with malignancy. The continued study of glutaminase and its activity is important for determining its role in pathologic conditions such as cancer.

About This Assay

Cayman's Glutaminase Activity Assay Kit provides a convenient method of detecting glutaminase activity in cell lysates and tissue homogenates. Measurement of glutaminase activity is carried out by the Glutamine/Glutamate Developer Enzyme Mix (see Figure 1), which detects levels of glutamate produced by glutaminase in the presence of MaxiProbe. The signal can be easily quantified by the absorbance at 570 nm. Under circumstances in which the glutaminase activity rate is limiting, the rate of increasing signal is directly proportional to the levels of glutaminase activity in the sample.

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

$$\text{L-Glutamate} + O_2 + H_2O \xrightarrow[\text{enzyme mix}]{\text{Developer}} \alpha\text{-KG} + NH_3 + H_2O_2$$

$$\text{MaxiProbe} + H_2O_2 \xrightarrow[\text{enzyme mix}]{\text{Developer}} \text{Compound detected at } A_{570}$$

Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Glutaminase Assay Buffer (5X) – (Item No. 400666)**

   This vial contains 10 ml of concentrated Glutaminase Assay Buffer. Dilute the contents of the vial with 40 ml of pure water to make Glutaminase Assay Buffer (1X). When stored at 4°C, this diluted buffer will be stable for at least two months.

2. **Glutaminase Substrate – (Item No. 400667)**

   This vial contains 500 µl of a glutamine solution. Prior to use in the assay, dilute 240 µl of the Glutaminase Substrate with 4,560 µl of Glutamine Assay Buffer (1X). This is a sufficient volume to assay 100 wells. Scale down as needed. The diluted substrate will be stable for four hours on ice. If all of the undiluted substrate will not be used at once, aliquot it and store at -20°C. Avoid more than three freeze-thaw cycles.

3. **Glutaminase Positive Control – (Item No. 400668)**

   This vial contains lyophilized glutaminase. Reconstitute the contents of the vial with 100 µl of Glutaminase Assay Buffer (1X). The reconstituted Glutaminase Positive Control will be stable for four hours on ice or two months when stored at -80°C. Avoid more than three freeze-thaw cycles.

4. **Glutamate Standard - (Item No. 400552)**

   This vial contains 50 µl of a 10 mM glutamate solution. If all of the Glutamate Standard will not be used at once, aliquot and store it at -20°C. Avoid more than three freeze-thaw cycles.

   Prior to each assay, add 20 µl of 10 mM Glutamate Assay Standard to 230 µl of Glutaminase Assay Buffer 1 (1X) to prepare an 800 µM bulk standard. Use this bulk standard in the preparation of the standards as shown on page 13.

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

   This vial contains a lyophilized mixture of enzymes. Reconstitute the contents of the vial with 300 µl of pure water and place it on ice. Do not vortex. One vial provides a sufficient volume to assay 100 wells. This will be used to make the Development Mix (see **Performing the Assay, on page 14**). The reconstituted Glutamine/Glutamate Developer Enzyme will be stable for two months when stored at -80°C. Avoid more than three freeze-thaw cycles.

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

   The vial contains 250 µl of MaxiProbe. This will be used to prepare the Development Mix (See **Performing the Assay, on page 14**). MaxiProbe will be stable for one hour at room temperature if capped and protected from light. If all of the MaxiProbe will not be used at one time, aliquot and store at -20°C. Avoid more than three freeze-thaw cycles.

Sample Preparation

NOTE: In order to express glutaminase levels as specific activity, it will be necessary to determine the protein concentration of the samples being tested. Protein Determination Kits are available for purchase from Cayman (Item Nos. 701780 | 704002).

Tissue Homogenate

1. Prior to dissection, rinse the tissue with PBS, pH 7.4, to remove any red blood cells and clots.
2. Homogenize the tissue with a Dounce homogenizer in 5-10 ml of cold Glutaminase Assay Buffer (1X) per gram of tissue. Alternatively, 100 mM Tris-HCl buffer, pH 7.5, or 100 mM potassium phosphate buffer, pH 7.5, can be used.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Transfer the supernatant to a new tube and discard the pellet. If not assaying on the same day, store the sample at -80°C.

Cell Lysate

1. Collect cells (~5 x 10^6) by centrifugation (*i.e.*, 1,000-2,000 x g for 10 minutes at 4°C). For adherent cells, use a rubber cell scraper instead of proteolytic enzymes.
2. Homogenize the cell pellet in 0.5-1 ml cold Glutaminase Assay Buffer (1X). Alternatively, 100 mM Tris-HCl buffer, pH 7.5, or 100 mM potassium phosphate buffer, pH 7.5, can be used.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Transfer the supernatant to a new tube and discard the pellet. If not assaying on the same day, store the sample at -80°C.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. It is recommended that two wells be designated for the Glutaminase Positive Control. It is suggested that each sample be assayed in duplicate and that the contents of each well are recorded on the template sheet provided on page 25. A typical layout of samples to be measured in duplicate is provided below. An optional background well can be set up for each sample (see Table 2 on page 15), although the background activity is typically insignificant.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	1	1	9	9	17	17	25	25	33	33
B	B	B	2	2	10	10	18	18	26	26	34	34
C	C	C	3	3	11	11	19	19	27	27	35	35
D	D	D	4	4	12	12	20	20	28	28	36	36
E	E	E	5	5	13	13	21	21	29	29	37	37
F	F	F	6	6	14	14	22	22	30	30	38	38
G	NC	NC	7	7	15	15	23	23	31	31	39	39
H	PC	PC	8	8	16	16	24	24	32	32	40	40

A-F = Standard A-F Wells
NC = Negative Control Wells
PC = Positive Control Wells
1-40 = Sample 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 of the assay is 100 µl in all of the wells.
- Use the diluted assay buffer in the assay.
- All reagents except the Glutaminase Positive Control and the Glutamine/Glutamate Developer Enzyme must be equilibrated to room temperature before beginning the assay. The enzymes must be placed on ice.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended to assay the samples in duplicate, but it is the user's discretion to do so.
- 40 samples can be assayed in duplicate. If adding sample background wells (see Step 2, Page 15), 20 samples can be assayed in duplicate.
- The assay is performed at room temperature.
- Monitor the absorbance at 570 nm.

Standard Curve Preparation

Use the 800 µM bulk standard (see **Reagent Preparation**, on page 8) to prepare the standards.

Label six clean test tubes A-F. Pipette 360 µl of Glutaminase Assay Buffer (1X) to tube A. Pipette 200 µl of Glutaminase Assay Buffer (1X) to tubes B-F. Transfer 40 µl of the bulk standard (800 µM) to tube A. Mix gently. Serially dilute the standard by transferring 200 µl from tube A to tube B. Mix gently. Next, transfer 200 µl from tube B to tube C. Mix gently. Repeat the process for tubes D-E. Do not add any standard to tube F. The diluted standards will be stable for at least one hour at room temperature.

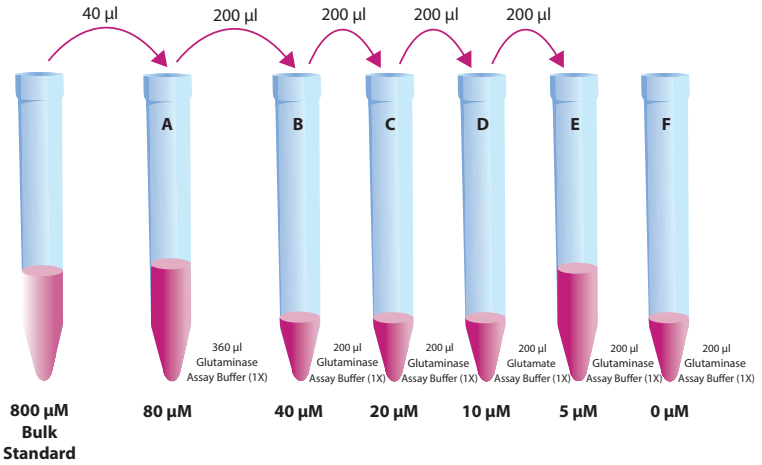


Figure 3. Preparation of the glutamate standards

Performing the Assay

1. **Prepare the Development Mix immediately prior to use:** Combine Glutaminase Assay Buffer (1X), reconstituted Glutamine/Glutamate Developer Enzyme, and MaxiProbe according to the table below. Scale as needed. The Development Mix will be stable for 15 minutes.

Reagent	50 Wells	100 Wells
Glutaminase Assay Buffer (1X)	2.88 ml	5.76 ml
Developer Enzyme	60 µl	120 µl
MaxiProbe	60 µl	120 µl
Total Volume:	3 ml	6 ml

Table 1. Development Mix preparation

2. Add the appropriate amount of reagent(s) to the designated wells as follows:

Reagent	Standard	Sample	Positive Control	Negative Control	Sample Background (optional)
Development Mix	50 µl	50 µl	50 µl	50 µl	50 µl
Standard	50 µl	--	--	--	--
Sample	--	10 µl	--	--	10 µl
Positive Control	--	--	10 µl	--	--
Glutaminase Assay Buffer (1X)	--	--	--	10 µl	40 µl

Table 2. Pipetting summary

3. Initiate the reactions by adding 40 µl of diluted substrate to all wells EXCEPT the standard wells and the optional sample background wells. Mix by gently pipetting.
4. Cover the plate with non-adhesive foil and incubate for 30 minutes at room temperature.
5. Remove the foil and immediately read the absorbance at 570 nm once every minute for 20 minutes.

ANALYSIS

Calculations

Plot the Standard Curve

1. Use the absorbance values of the last time point to determine the average absorbance (A_{570}) of the standards.
2. Plot the average absorbance values (from step 1 above) of each standard as a function of glutamate concentration.
3. Obtain the slope ($A_{570}/\mu M$) of the linear curve. See Figure 5 on page 18 for a typical standard curve.

Determine Glutaminase Activity

1. Determine the change in absorbance per minute (ΔA_{570}/min) for each sample and control:
   A. Plot the absorbance values as a function of time. An example is shown in Figure 4 on page 17.
   B. Obtain the rate, which corresponds to the slope of the linear portion of the curve.
2. Determine the average rate of each sample and control.
3. Calculate the glutaminase activity (U/ml) using the equation below. One unit is defined as the amount of enzyme that catalyzes the conversion of 1 nmol of glutamine to glutamate per minute under the specified conditions of this assay.

$$\text{activity (nmol/min/ml)} = \left[\frac{\text{sample rate } (\Delta A_{570}/\text{min})}{\text{standard curve slope } (A_{570}/\mu M)}\right] \text{X sample dilution} = \text{U/ml}$$

4. **Optional:** If the protein concentration of the samples has been determined, specific activity can be calculated using the equation below.

$$\text{specific activity } \left(\frac{U}{mg}\right) = \frac{\text{activity (U/ml)}}{\text{sample protein concentration (mg/ml)}}$$

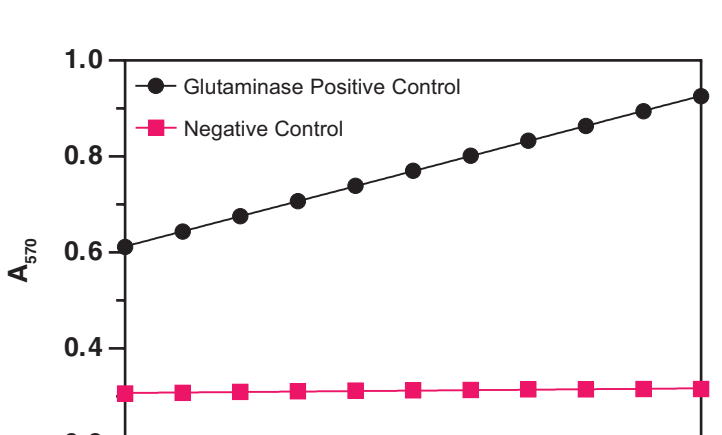


Figure 4. Activity of the Glutaminase Positive Control

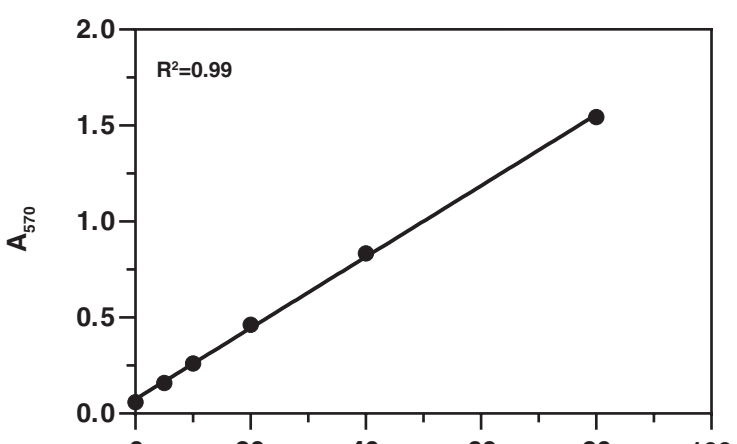


Figure 5. Glutamate standard curve

Performance Characteristics

Sensitivity:

The limit of detection for this assay is 0.09 U/ml.

Precision:

When a series of 24 measurements of mouse kidney sample were performed on the same day, the intra-assay coefficient of variation was 2%. When a series of five mouse kidney sample measurements were performed on two different days under the same experimental conditions, the inter-assay coefficient of variation was 7%.

Spike and Recovery

Rat kidney tissue samples were spiked with glutaminase and analyzed using the Glutaminase Activity Assay Kit. The results are shown below.

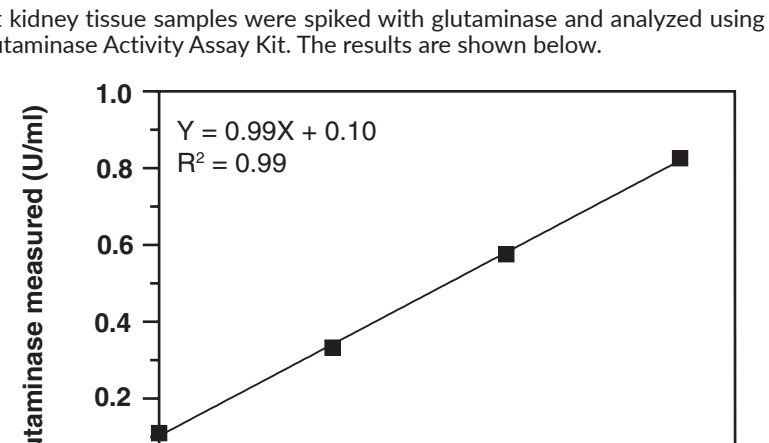


Figure 6. Spike and recovery of glutaminase in rat kidney tissue

Interferences

The following reagents were tested for interference in the assay.

Reagent		Will Interfere
Buffers	T-PER	Yes
	M-PER	Yes
	RIPA	Yes

Table 3. Interferences

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	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 activity detected in samples (*i.e.*, ΔA_{570}/min <0.001	A. If the samples' A_{570} >>> negative control's, the reaction is complete indicating that the glutaminase levels are too high B. If A_{570} of the samples at t=20 min ≈ negative control, the glutaminase activity is too low C. There is no glutaminase in the sample	A. Dilute the samples further with Assay Buffer (1X) and re-assay B. Re-assay using a lower dilution C. Re-assay to verify
No change observed in A_{570} over time in positive control wells	A. The Development Mix was prepared incorrectly B. The Glutaminase Positive Control and/or the diluted substrate were not added to the wells	A. Freshly prepare the Development Mix and re-assay B. Re-assay with the positive control and/or the diluted substrate added to the designated wells

Item No.	Reagent	Procedure
400666	Glutaminase Assay Buffer (5X)	Dilute to 1X with pure water
400667	Glutaminase Substrate	Dilute 1:20 with Assay Buffer (1X) prior to each assay
400668	Glutaminase Positive Control	Reconstitute with 100 µl Assay Buffer (1X)
400552	Glutamate Standard	Dilute 1:12.5 with Assay Buffer (1X) prior to preparing standards (see Figure 3 on page 13)
400615	Glutamine/Glutamate Developer Enzyme Mix	Reconstitute with 300 µl pure water
400610	MaxiProbe	Ready to use as supplied

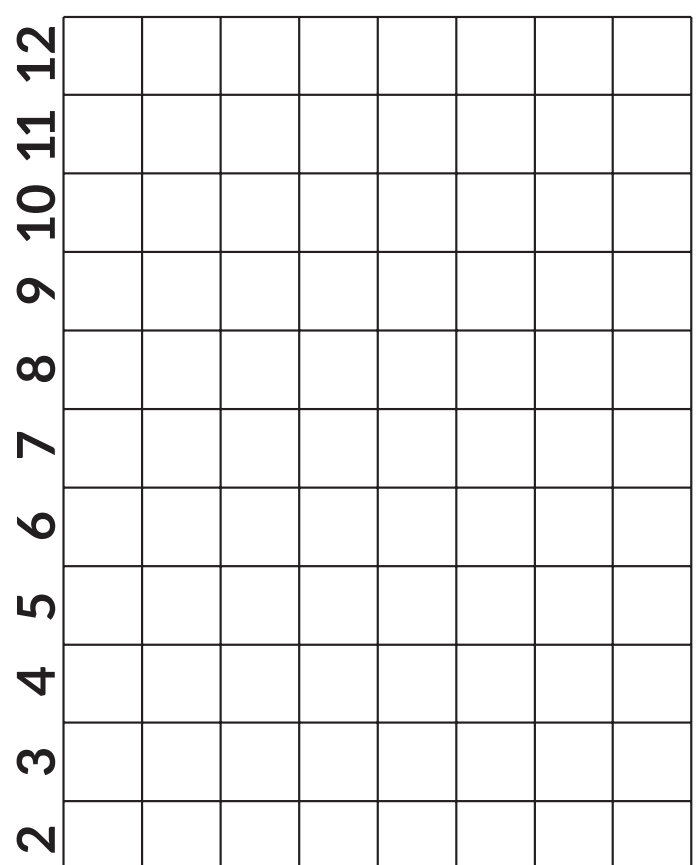
Table 4. Reagent preparation summary

	Standard Wells	Sample Wells	Positive Control Wells	Negative Control Wells	Sample Background Wells
Prepare Development Mix: Mix Assay Buffer (1X), Developer Enzyme Mix, and MaxiProbe at a 48:1:1 ratio					
Add Development Mix	50 µl	50 µl	50 µl	50 µl	50 µl
Add standards	50 µl	--	--	--	--
Add sample	--	10 µl	--	--	10 µl
Add reconstitued Glutaminase Positive Control	--	--	10 µl	--	--
Add Glutaminase Assay Buffer (1X)	--	--	--	10 µl	40 µl
Add diluted substrate	--	40 µl	40 µl	40 µl	--
Cover with foil and incubate for 30 minutes at room temperature					
Read: A_{570} every minute for 20 minutes immediately after incubation					

Table 5. Assay summary

References

1. Curthoys, N.P. and Watford, M. Regulation of glutaminase activity and glutamine metabolism. *Annu. Rev. Nutr.* **15**, 133-159 (1995).
2. Campos-Sandoval, J.A., Martín-Rufián, M., Cardona, C. *et al.* Glutaminases in brain: Multiple isoforms for many purposes. *Neurochem. Int.* **88**, 1-5 (2015).
3. Buczkowska, J. and Szeliga, M. Two faces of glutaminase GLS2 in carcinogenesis. *Cancers* **15(23)**, 5566 (2023).



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.