ab197011 – Glutamine Assay Kit (Colorimetric)

For rapid, sensitive and accurate measurement of Glutamine levels in various samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab197011 (use http://www.abcam.cn/ab197011 for China, or http://www.abcam.co.jp/ab197011 for Japan)

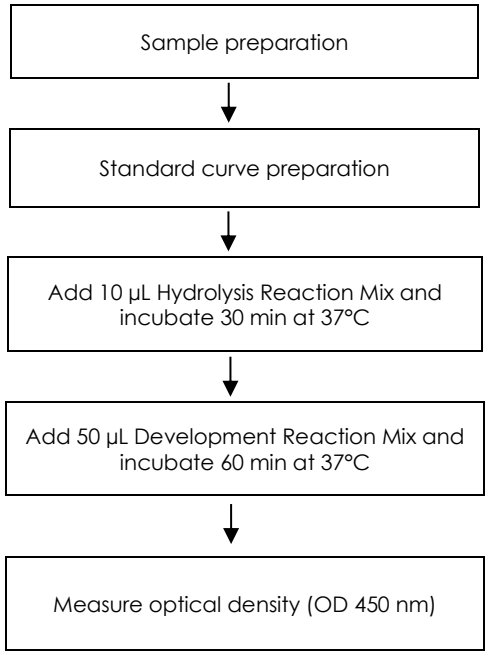
Background:

Glutamine Assay Kit (ab197011) provides a simple, rapid and reliable procedure for quantifying glutamine in tissue, urine, plasma, serum, and other biological fluids.

The assay is based on the hydrolysis of Glutamine to Glutamate producing a stable signal, which is directly proportional to the amount of Glutamine (Gln) in the sample. The Glutamate is used in downstream reactions to ultimately produce a stable chromophore, detectable by absorbance at 450 nm. The assay can detect as little as 25 µM of Gln in a variety of biological samples.

Assay Summary:

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.



QUICK ASSAY PROCEDURE

- Preheat incubator and/or plate reader to 37°C
- Solubilize Enzyme Mix VIII, thaw Developer Solution III, Assay Buffer 31, Glutamine Standard, Assay Buffer 29 and Hydrolysis Enzyme Mix II (aliquot if necessary
- Prepare appropriate standard curve
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard and samples (40 µL).
- Prepare and add 10 µL Hydrolysis Enzyme Reaction Mix to each well (Number samples + standards + 1).
- Incubate 30 min at 37°C
- Prepare and add 10 µL Hydrolysis Reaction Mix to each well
- Incubate plate at 37°C for 30 mins.
- Prepare and add 50 µL Development Reaction Mix to each well.
- Incubate plate at 37°C for 60 mins.
- Measure plate at OD 450 nm

Precautions & Limitations:

Please read these instructions carefully prior to beginning the assay.
All kit components have been formulated and quality control tested to function successfully as a kit.

- Modifications to the kit components or procedures may result in loss of performance.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

Storage and Stability:

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
Developer Solution III	1 Each	-20°C	-20°C
Assay Buffer 31	25 mL	-20°C	-20°C
Enzyme Mix VIII	1 Each	-20°C	-20°C
Glutamine Standard	1 Each	-20°C	-20°C
Assay Buffer 29	25 mL	-20°C	-20°C
Hydrolysis Enzyme Mix II	1 Each	-20°C	-20°C

PLEASE NOTE: Developer Solution III was previously labeled as Developer, and Assay Buffer 31 as Assay Buffer XXXI and Development Buffer. Also, Enzyme Mix VIII was previously labeled as Development Enzyme Mix (lyophilized), and Assay Buffer 29 as Assay Buffer XXIX and Hydrolysis Buffer, and Hydrolysis Enzyme Mix II as Hydrolysis Enzyme Mix. The composition has not changed.

Materials Required, Not Supplied:

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance (OD) at 450 nm (colorimetric)
- 96 well clear plate with clear flat bottom (colorimetric assay)
- Orbital shaker
- 10K Spin Column
- Multi-well spectrophotometer
- Microcentrifuge
- Dounce homogenizer (if using tissue)
- 1 x PBS, pH 7.4
- MilliQ water or other type of double distilled/deionized water (ddH_2O)

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

Assay Buffer 29 and Assay Buffer 31: Bring to room temperature before use. Store at -20°C. Stable for two months.

Hydrolysis Enzyme Mix II: Reconstitute with 200 µL Assay Buffer 29 to make the stock solution. Pipette gently to dissolve. Store at -20°C. Keep on ice while in use. Stable for two months.

Enzyme Mix VIII: Reconstitute with 200 µL Assay Buffer 31 to make the stock solution. Pipette gently to dissolve. Store at -20°C. Keep on ice while in use. Stable for two months.

Development Solution III: Reconstitute with 200 µL Assay Buffer 31 to make the stock solution. Pipette gently to dissolve. Store at -20°C. Keep on ice while in use. Stable for two months.

Glutamine standard: Reconstitute with 100 µL ddH_2O to generate 10 mM solution. Store at -20°C. Stable for two months.

Sample Preparation:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

1. We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
2. Interferences:
   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 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 Glutamine in the test samples with a low concentration of Glutamine, we recommend spiking samples with a known amount of Glutamine Standard (6 nmol).

Tissue Samples:

1. Harvest the amount of tissue necessary for each assay (initial recommendation = 10-20 mg.
2. Wash tissue in cold PBS.
3. Homogenize tissue in 10x (w/v) Assay Buffer 29 with a Dounce homogenizer sitting on ice, with 10 - 15 passes.
4. Centrifuge samples at 10,000 x g for 10 minutes at 4°C to remove any insoluble material.
5. Collect supernatant and transfer to a clean tube.
6. Keep on ice.

Liquid Samples (Urine and other biological fluids):

1. Centrifuge biological samples for 10 minutes at 10,000 x g at 4°C .
2. Collect supernatant and transfer to a clean tube.
3. Keep on ice.

Initial sample recommendation = 1 - 40 µL/well

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

Prepare I mM dilution of Glutamine Standard as follows:

1. 1 mM dilution: Add 10 µL of 10 mM Glutamine Standard to 90 µL ddH_2O. Gently pipette up and down a few times to ensure all standard is removed from tip. Mix well by inversion.

   Add 0, 2, 4, 6, 8 and 10 µl of Glutamine 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 or prepare the standard curve dilution as described in the table below in a microplate or microcentrifuge tubes (sufficient for duplicate standard curves).

Standard #	Volume of 1 mM Standard (µL)	ddH_2O (µL)	Final volume standard in well (µL)	Glutamine Amount (nmol/well)
1	0	100	40	0
2	5	95	40	2
3	10	90	40	4
4	15	85	40	6
5	20	80	40	8
6	25	75	40	10

Assay Procedure:

- Keep enzymes and heat labile components and samples on ice during the assay.
- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls, and samples in duplicate.

1. Set up Reaction wells:
   - Standard wells = 40 µL standard dilutions.

- Sample wells = 1 – 40 µL samples (adjust volume to 40 µL/well with ddH_2O).
- Paired Sample Background wells = 1 – 40 µL samples (adjust volume to 40 µL/well with ddH_2O).

2. Each well (standards, samples, and controls) requires 10 µL of Hydrolysis Reaction Mix as shown in the table below. To ensure consistency, use the tables below to prepare a Master Mix of the appropriate Reaction Mix for your assay using the following calculation:
   X µL component x (Number reactions +1).

Hydrolysis Reaction Mix:

1. Add 2 µL Hydrolysis Enzyme Mix II and 8 µL of Assay Buffer 29 to each Standard and Sample wells as shown in table below. Do not add Hydrolysis enzyme to Sample Background wells.

Component	Standard/Sample wells (µL)	Sample Background wells (µL)
Hydrolysis Enzyme Mix II	2	0
Assay Buffer 29	8	10

2. Mix well. Incubate for 30 min at 37°C.
   - *For samples having high glutamate levels, add 10 µL of Assay Buffer 29 to sample background control well(s). Final volume should be 50 µL/well. Incubate for 30 min at 37°C alongside sample wells .*

Development Reaction Mix:

1. Mix enough reagents for the number of assays to be performed (X µL component x (Number reactions +1). For each well, prepare 50 µL Reaction Mix containing:

Component	Reaction Mix (µL)
Assay Buffer 31	46
Enzyme Mix VIII	2
Developer Solution III	2

3. Mix Master Reaction Mix by inversion. Add 50 µL of the Master Reaction Mix to each well. Use a clean tip for each well.
4. Mix and incubate at 37°C for 60 minutes, protected from light.
5. Measure output on a microplate reader at OD 450 nm for Colorimetric assay.

Calculations:

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean absorbance value of the blank (Standard #1) from all standard. This is the corrected absorbance.
3. Plot the corrected absorbance values for each standard as a function of the final amount of Glutamine (nmol).
4. Calculate the equation of the standard curve using a linear regression and determine the slope.
5. Subtract the background absorbance from the Sample absorbance. This is the corrected sample absorbance.
   Note: If background was not performed, subtract the mean absorbance value of the blank (Standard #1).
6. Interpolate sample readings from the standard curve plotted using the following equation:

$$B = \left(\frac{Corrected\ sample\ absorbance - (y - intercept)}{Slope}\right)$$

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

$$B = \frac{Corrected\ Absorbance_{Sample}}{Corrected\ Absorbance_{\ Sample+Gln\ Spike} - Corrected\ Absorbance_{Sample}} \times\ Gln\ Spike\ (nmol)$$

Note: If following the spike recommendations in sample prep 2d, the glutamine spike is 6 nmoles.

7. Concentration of Glutamine in the test samples is calculated as:

$$Sample\ Glutamine\ Concentration\ (C) = \frac{B}{V} \times D = nmol/\mu L\ \ or\ mM$$

Where:
B = amount of Glutamine in the sample well calculated from standard curve (in nmoles).
V= amount of sample volume added in sample wells (in µL).
D = sample dilution factor (before adding to the well).

Note: Glutamine concentration can also be expressed as nmol/mg of protein or nmol/mg of creatinine in case of urine.
Note: Glutamine molecular weight: 146.1 g/mol

Technical Hints

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

For technical or commercial enquiries please go to:
www.abcam.com/contactus
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