ab197011 – Glutamine Assay Kit (Colorimetric)

For the quantitative measurement of Gln in various fluids and tissues.
For research use only - not intended for diagnostic use.
PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab197011

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Developer Solution III/Developer	1 vial	-20°C
Assay Buffer XXXI/Development Buffer	25 mL	-20°C
Enzyme Mix VIII/Development Enzyme Mix (lyophilized)	1 vial	-20°C
Glutamine Standard	1 vial	-20°C
Assay Buffer XXIX/Hydrolysis Buffer	25 mL	-20°C
Hydrolysis Enzyme Mix II/Hydrolysis Enzyme Mix	1 vial	-20°C

Materials Required, Not Supplied

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

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- 10K Spin Column

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Assay Buffer XXIX/Hydrolysis Buffer and Assay Buffer XXXI/Development Buffer: Bring to room temperature before use. Store at -20°C. Stable for two months.

Hydrolysis Enzyme Mix II/Hydrolysis Enzyme Mix: Reconstitute with 220 µl Assay Buffer XXIX/Hydrolysis Buffer 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/Development Enzyme Mix: Reconstitute with 220 µl Assay Buffer XXXI/Development Buffer. Pipette gently to dissolve. Aliquot & store at -20°C. Keep on ice while in use. Stable for two months.

Development Solution III/Developer: Reconstitute with 220 µl Assay Buffer XXXI/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.

Assay Protocol

Sample Preparation:

1. 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.
2. For mammalian tissues, homogenize ~10-20 mg of tissue on ice using 10x (v/w) Assay Buffer XXIX/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.

Δ 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).

Standard Curve Preparation:

1. Dilute Gln Standard to 1 mM by adding 10 µl of 10 mM Gln Standard to 90 µl of ddH_2O.
2. 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.
3. Adjust the volume to 40 µl/well with ddH_2O.

Hydrolysis Mix:

1. Add 2 µl Hydrolysis Enzyme Mix II/Hydrolysis Enzyme mix to the Standard and Sample wells as follows:

Item	Standard/Sample	* Sample Background
Hydrolysis Enzyme Mix II/Hydrolysis Enzyme Mix	2 µl	-
Assay Buffer XXIX/Hydrolysis Buffer	8 µl	10 µl

2. 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 Assay Buffer XXIX/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.*

Reaction Mix:

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

Item	Reaction Mix
Assay Buffer XXXI/Development Buffer	46 µl
Enzyme Mix VIII/Development Enzyme Mix	2 µl
Development Solution III/Developer	2 µl

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

Measurement

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

Calculation

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

$$\textbf{Sample Gln concentration (C)} = \frac{B}{V} \times \mathbf{D\ nmol/\mu l\ or\ mM}$$

Where: **B** = Amount of Gln in the sample well from Standard Curve (nmol)
V = Sample volume added into the reaction well (µL)
D = Sample dilution factor

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

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

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

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)