ab197011
Glutamine Assay Kit
(Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Glutamine in various biological samples.

This product is for research use only and is not intended for diagnostic use.
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INTRODUCTION

1. BACKGROUND

Glutamine Colorimetric Assay kit (ab197011) is a simple and sensitive assay that detects the biologically relevant concentrations of glutamine (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 glutamine in the sample. The assay can detect as little as 25 μM of glutamine in a variety of biological samples.

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 a 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. Assay of nicotinamide nucleotides is of continual interest in the studies of energy transforming and redox state of cells or tissues.
2. **ASSAY SUMMARY**

- Standard curve preparation

- Sample preparation*

- Add hydrolysis mix and incubate 37°C 30 min

- Add reaction mix and incubate 37°C 60 min

- Measure optical density (OD450 nm)

*Samples might require deproteinization.
3. **PRECAUTIONS**

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.

4. **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.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**
5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
<th>Storage Condition (After Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis Buffer</td>
<td>25 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Development Buffer</td>
<td>25 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Hydrolysis Enzyme Mix (lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Development Enzyme Mix (lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Developer (lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Glutamine Standard (Gln) (lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

6. **MATERIALS REQUIRED, NOT SUPPLIED**

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

- MilliQ water or other type of double distilled water (ddH₂O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD 450 nm
- 96 well plate: clear plates (flat bottom) for colorimetric assay
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)

For deproteinization step, additional reagents are required:

- Perchloric acid (PCA) 4M, ice cold
- Potassium Hydroxide (KOH) 2M
- 10 kD Spin Columns (ab93349) – for fluid samples, if not performing PCA precipitation
7. **LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- 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.
8. **TECHNICAL HINTS**

- This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

- Keep enzymes and heat labile components and samples on ice during the assay.

- Make sure all buffers and developing solutions are at room temperature before starting the experiment.

- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.

- Avoid foaming or bubbles when mixing or reconstituting components.

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.

- Ensure plates are properly sealed or covered during incubation steps.

- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.

- Make sure you have the appropriate type of plate for the detection method of choice.

- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.
9. **REAGENT PREPARATION**

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Hydrolysis Buffer:**

   Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C. Use within 2 months.

9.2 **Development Buffer:**

   Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C. Use within 2 months.

9.3 **Hydrolysis Enzyme Mix:**

   Reconstitute in 220 µL Hydrolysis Buffer to make stock solution. Pipette gently to dissolve. Aliquot enzyme so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.4 **Development Enzyme Mix:**

   Reconstitute in 220 µL Development Buffer. Pipette gently to dissolve. Aliquot enzyme so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.5 **Developer:**

   Reconstitute in 220 µL Development Buffer. Pipette gently to dissolve. Aliquot developer so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.6 **Glutamine Standard (Gln):**

   Reconstitute in 100 µL of ddH₂O to generate a 10 mM standard stock solution. Aliquot standard so that you have enough volume to perform the desired number of tests. Store at -20°C. Keep on ice while in use.
10. **STANDARD PREPARATION**

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

10.1 Prepare a 1 mM Glutamine standard by diluting 10 µL of the reconstituted Glutamine standard with 90 µL of ddH₂O.

10.2 Using 1 mM Glutamine standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Volume of Standard (µL)</th>
<th>ddH₂O (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End Conc. Glutamine in well</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>120</td>
<td>40</td>
<td>0 nmol/well</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>114</td>
<td>40</td>
<td>2 nmol/well</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>108</td>
<td>40</td>
<td>4 nmol/well</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>102</td>
<td>40</td>
<td>6 nmol/well</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>96</td>
<td>40</td>
<td>8 nmol/well</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>90</td>
<td>40</td>
<td>10 nmol/well</td>
</tr>
</tbody>
</table>

Each dilution has enough amount of standard to set up duplicate readings (2 x 40 µL).
11. **SAMPLE PREPARATION**

**General Sample information:**

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- 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 as well as the Deproteinization step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples 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.

11.1 **Cell (adherent or suspension) samples:**

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of ice cold Hydrolysis Buffer.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Centrifuge sample for 10 minutes at 4°C at 10,000 x g using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.1.7 Keep on ice.

11.1.8 Perform deproteinization step as described in section 11.4.

11.2 **Tissue samples:**

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 -20 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 10X (v/w) of ice cold Hydrolysis Buffer.
11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.5 Centrifuge samples for 10 minutes at 4°C at 10,000 x g using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

11.2.7 Keep on ice.

11.2.8 Perform deproteinization step as described in section 11.4.

11.3 Plasma, Serum and Urine and other biological fluids:

11.3.1 Centrifuge biological fluids at 4°C at 10,000 x g using a cold microcentrifuge to remove any insoluble material.

11.3.2 Collect supernatant and transfer to a clean tube.

Plasma, serum and urine samples generally contain high amount of proteins, so they should be deproteinized as described in section 11.4.

Alternatively, you can use 10kD Spin column (ab93349) to deproteinize biological fluids.

To find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (1/2 – 1/5 – 1/10).

11.4 Deproteinization step:

Prepare samples as specified in protocol. You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.

11.4.1 Add ice cold PCA 4 M to a final concentration of 1 M in the homogenate solution and vortex briefly to mix well. **NOTE:** high protein concentration samples might need more PCA.

11.4.2 Incubate on ice for 5 minutes.
11.4.3 Centrifuge samples at 13,000 x g for 2 minutes at 4°C in a cold centrifuge and transfer supernatant to a fresh tube. Measure volume of supernatant.

11.4.4 Precipitate excess PCA by adding an equal volume of ice-cold 2 M KOH to supernatant obtained in previous step and vortex briefly. This will neutralize the sample and precipitate excess PCA. After neutralization, it is very important that pH equals 6.5 – 8 (use pH paper to test 1 µL of sample). Any left over PCA will interfere with the assay.

11.4.5 Centrifuge at 13,000 x g for 15 minutes at 4°C and collect supernatant.

Samples are now deproteinized, neutralized and PCA has been removed. The samples are now ready to use in the assay.

**Sample Recovery**

The deproteinized samples will be diluted from the original concentration.

To calculate the dilution factor of your final sample, simply apply the following formula:

\[
\% \text{ original concentration} = \frac{\text{initial sample vol}}{(\text{initial sample vol} + \text{vol PCA added} + \text{vol KOH added})} \times 100
\]

**NOTE:** We suggest using different volumes of sample to ensure readings are within the Standard Curve range.

Endogenous compounds may interfere with the assay. To ensure accurate determination of glutamine in the test samples or for samples having low concentration of glutamine, we recommend spiking samples with a known amount of Glutamine Standard (6 nmol).
12. ASSAY PROCEDURE and DETECTION

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

12.1 Set up Reaction wells:
- Standard wells = 40 µL standard dilutions.
- Sample wells = 1 – 40 µL samples (adjust volume to 40 µL/well with ddH₂O).
- Background control sample wells = 1 – 40 µL samples (adjust volume to 40 µL/well with ddH₂O). **NOTE:** for samples with high levels of glutamate, as they can generate high background.

12.2 Hydrolysis Mix:
Add 2 µL Hydrolysis Mix to the standard and sample wells as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Hydrolysis Mix (µL)</th>
<th>Background Hydrolysis Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis Buffer</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Hydrolysis Enzyme Mix</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Mix enough reagents for the number of assays (samples, standards) to be performed. Prepare a master mix of the Hydrolysis Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards + 1).

12.3 Add 10 µL of Hydrolysis Mix into each standard and sample wells.
12.4 Add 10 µL of Background Hydrolysis Mix into each background control sample wells.
12.5 Incubate standard, samples and background control samples with the correspondent Hydrolysis Mix for 30 minutes at 37°C.
12.6 **Glutamine Reaction Mix:**

Prepare 50 µL of Glutamine Reaction Mix for each reaction:

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development Buffer</td>
<td>46</td>
</tr>
<tr>
<td>Development Enzyme Mix</td>
<td>2</td>
</tr>
<tr>
<td>Developer</td>
<td>2</td>
</tr>
</tbody>
</table>

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

\[ X \, \mu L \, \text{component} \times (\text{Number samples} + \text{Standards} + \text{background control sample} + 1) \]

12.7 Add 50 µL of Glutamine Reaction Mix into each standard, sample and background control sample well.

12.8 Mix well and incubate at 37°C for 60 minutes protected from light.

12.9 Measure absorbance at OD=450 nm on a microplate reader.
13. **CALCULATIONS**

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of glutamine.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Extrapolate sample readings from the standard curve plotted using the following equation:

\[
B = \left(\frac{\text{Corrected absorbance} - (y - \text{intercept})}{\text{Slope}}\right)
\]

13.7 Concentration of glutamine (nmol/µL or mM) in the test samples is calculated as:

\[
\text{Glutamine concentration} = \left(\frac{B}{V}\right) \times D
\]
DATA ANALYSIS

Where:

B = Amount of glutamine in the sample well (nmol).
V = Sample volume added into the reaction well (µL).
D = Sample dilution factor.

Glutamine molecular weight: 146.1 g/mol.

Glutamine concentration can also be expressed as nmol/mg of protein or nmol/mg of creatinine in case of urine.

13.8 For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, the concentration of glutamine in sample well is calculated as:

\[
Gln = \left( \frac{ODs_{\text{cor}}}{ODs + Ts_{\text{cor}} - (ODs_{\text{cor}})} \right) \times Gln \text{ spike (nmol)}
\]

Where:

ODs cor = OD sample corrected
ODs = OD sample
Ts cor = Gln amount from standard curve corrected
14. **TYPICAL DATA**

**TYPICAL STANDARD CURVE** – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

![Typical Glutamine Standard calibration curve using colorimetric reading.](image)

**Figure 1.** Typical Glutamine Standard calibration curve using colorimetric reading.
Figure 2: Measurement of glutamine concentration in Human urine (5 µL).

Figure 3: Samples were deproteinized prior measurement and spiked with known amount of glutamine (6 nmol).
15. **QUICK ASSAY PROCEDURE**

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

- Prepare buffers, standard, Hydrolysis enzyme mix, Development enzyme mix and developer (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings), including deproteinization step.
- Set up plate for standard (40 µL), samples (40 µL) and background control sample wells (40 µL).
- Prepare 1:10 dilution of Hydrolysis enzyme mix stock.
- Prepare Hydrolysis Mix and Background Hydrolysis Mix.

<table>
<thead>
<tr>
<th>Component</th>
<th>Hydrolysis Mix (µL)</th>
<th>Background Hydrolysis Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis Buffer</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Diluted Hydrolysis Enzyme Mix</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

- Add 10 µL Hydrolysis Mix to standard and samples.
- Add 10 µL Background Hydrolysis Mix to background sample control wells.
- Incubate plate 37°C 30 minutes.
- Prepare Glutamine Reaction Mix (Number samples + standards + background sample control wells + 1).

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development Buffer</td>
<td>46</td>
</tr>
<tr>
<td>Development Enzyme Mix</td>
<td>2</td>
</tr>
<tr>
<td>Developer</td>
<td>2</td>
</tr>
</tbody>
</table>
- Add 50 µL of Glutamine Reaction Mix to the standard, sample and background control sample wells.
- Incubate plate 37°C 60 minutes protected from light.
- Measure plate at OD450 nm.
## 16. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Use of ice-cold buffer</td>
<td>Buffers must be at room temperature</td>
</tr>
<tr>
<td></td>
<td>Plate read at incorrect wavelength</td>
<td>Check the wavelength and filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>Use of a different 96-well plate</td>
<td>Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate</td>
</tr>
<tr>
<td>Sample with erratic readings</td>
<td>Samples not deproteinized (if indicated on protocol)</td>
<td>Use PCA precipitation protocol for deproteinization</td>
</tr>
<tr>
<td></td>
<td>Cells/tissue samples not homogenized completely</td>
<td>Use Dounce homogenizer, increase number of strokes</td>
</tr>
<tr>
<td></td>
<td>Samples used after multiple free/thaw cycles</td>
<td>Aliquot and freeze samples if needed to use multiple times</td>
</tr>
<tr>
<td></td>
<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use</td>
</tr>
<tr>
<td></td>
<td>Presence of interfering substance in the sample</td>
<td>Check protocol for interfering substances; deproteinize samples</td>
</tr>
<tr>
<td>Lower/Higher readings in samples and Standards</td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
</tr>
<tr>
<td></td>
<td>Allowing reagents to sit for extended times on ice</td>
<td>Always thaw and prepare fresh reaction mix before use</td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Verify correct incubation times and temperatures in protocol</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>--------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Standard readings do not follow a linear pattern</td>
<td>Pipetting errors in standard or reaction mix</td>
<td>Avoid pipetting small volumes (&lt; 5 µL) and prepare a master mix whenever possible</td>
</tr>
<tr>
<td></td>
<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the tubes</td>
</tr>
<tr>
<td></td>
<td>Standard stock is at incorrect concentration</td>
<td>Always refer to dilutions on protocol</td>
</tr>
<tr>
<td>Unanticipated results</td>
<td>Measured at incorrect wavelength</td>
<td>Check equipment and filter setting</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>Troubleshoot if it interferes with the kit</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/ below the linear range</td>
<td>Concentrate/ Dilute sample so it is within the linear range</td>
</tr>
</tbody>
</table>
17. FAQ
18. **INTERFERENCES**

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Glutamate.