Glutaminase Microplate Assay Kit
User Manual

Catalog # CAK1065

Detection and Quantification of Glutaminase (GLS) Activity in Tissue extracts, Cell lysate Samples.

For research use only. Not for diagnostic or therapeutic procedures.
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I. INTRODUCTION

Glutaminase (glutamine aminohydrolase or GLS) catalyzes the following reaction: Glutamine + H2O → Glutamate + NH3. The enzyme has tissue-specific roles in multiple organs. Two different mammalian phosphate-activated GLS isoforms are known: GLS1 (kidney-type) and GLS2 (liver-type; a target of the tumor suppressor protein p53). The hydrolytic activity of glutaminase generates ammonia for urea synthesis in the liver similar to that mediated by glutamate dehydrogenase. During renal acidosis, glutaminase is induced in the kidney, leading to increased excretion of ammonia, which plays an important role in maintaining acid-base homeostasis.

Glutaminase regulates the levels of the neurotransmitter glutamate in the brain. The rate of glutaminolysis is known to increase in tumors and may be a hot spot for regulation of cancer cell metabolism. Inhibitors of GLS may therefore be candidate drugs for cancer therapy.

The assay is initiated with the enzymatic hydrolysis of glutamine by GLS. The enzyme catalysed reaction products can be measured at a colorimetric readout at 420 nm.
II. KIT COMPONENTS

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-Well Microplate</td>
<td>1 plate</td>
<td></td>
</tr>
<tr>
<td>Assay Buffer</td>
<td>30 ml x 4</td>
<td>4 °C</td>
</tr>
<tr>
<td>Substrate</td>
<td>Powder x 1</td>
<td>4 °C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>30 ml x 1</td>
<td>RT</td>
</tr>
<tr>
<td>Reaction Buffer</td>
<td>5 ml x 1</td>
<td>RT</td>
</tr>
<tr>
<td>Dye Reagent</td>
<td>2 ml x 1</td>
<td>RT</td>
</tr>
<tr>
<td>Standard (1 μmol/ml)</td>
<td>1 ml x 1</td>
<td>4 °C</td>
</tr>
</tbody>
</table>

Note:

**Substrate**: add 20 ml distilled water to dissolve before use, store at 4 °C.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 420 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice
IV. SAMPLE PREPARATION

1. For cell and bacteria samples
Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for $5 \times 10^6$ cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples
Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.
V. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Sample</th>
<th>Control</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>20 μl</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Distilled water</td>
<td>--</td>
<td>20 μl</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Substrate</td>
<td>200 μl</td>
<td>200 μl</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Mix, put it in the oven, 37 °C for 1 hour.

| Stop Solution | 300 μl | 300 μl | -- | -- |

Mix, wait for 10 minutes, centrifuged at 8,000g, 4 °C for 10 minutes, add the supernatant into the microplate.

| Supernatant   | 130 μl | 130 μl | -- | -- |
| Standard      | --     | --     | 130 μl | -- |
| Distilled water | --     | --     | -- | 130 μl |
| Reaction Buffer | 50 μl  | 50 μl  | 50 μl | 50 μl |
| Dye Reagent   | 20 μl  | 20 μl  | 20 μl | 20 μl |

Mix, record absorbance measured at 420 nm in 2 minutes.
VI. CALCULATION

Unit Definition: one unit is defined as the enzyme that generates 1 μmol of ammonia per hour.

1. According to the protein concentration of sample
GLS (U/mg) = \( C_{\text{standard}} \times \frac{(OD_{\text{Sample}} - OD_{\text{Control}})}{(OD_{\text{Standard}} - OD_{\text{Blank}})} \times \frac{1}{C_{\text{protein}}} \times 4 \)

\[ = 4 \times \frac{(OD_{\text{Sample}} - OD_{\text{Control}})}{(OD_{\text{Standard}} - OD_{\text{Blank}})} \times \frac{1}{C_{\text{protein}}} \]

2. According to the weight of sample
GLS (U/g) = \( C_{\text{standard}} \times \frac{(OD_{\text{Sample}} - OD_{\text{Control}})}{(OD_{\text{Standard}} - OD_{\text{Blank}})} \times \frac{1}{(V_{\text{Sample}} \times W / V_{\text{Assay}})} \times 4 \)

\[ = 200 \times \frac{(OD_{\text{Sample}} - OD_{\text{Control}})}{(OD_{\text{Standard}} - OD_{\text{Blank}})} \times \frac{1}{W} \]

3. According to the quantity of cells or bacteria
GLS (U/10⁴) = \( C_{\text{standard}} \times \frac{(OD_{\text{Sample}} - OD_{\text{Control}})}{(OD_{\text{Standard}} - OD_{\text{Blank}})} \times \frac{1}{(V_{\text{Sample}} \times N / V_{\text{Assay}})} \times 4 \)

\[ = 200 \times \frac{(OD_{\text{Sample}} - OD_{\text{Control}})}{(OD_{\text{Standard}} - OD_{\text{Blank}})} \times \frac{1}{N} \]

\( C_{\text{protein}} \): the protein concentration, mg/ml;
\( C_{\text{standard}} \): the concentration of Standard, 1 μmol/ml;
\( W \): the weight of sample, g;
\( N \): the quantity of cell or bacteria, N \times 10⁴;
\( V_{\text{sample}} \): the volume of sample, 0.02 ml;
\( V_{\text{assay}} \): the volume of Assay buffer in sample preparation, 1 ml;
\( T \): the reaction time, 1 hour;
VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.

![Standard curve graph](image)

Detection Range: 10 μmol/L - 500 μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to
www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES