Detection and Quantification of Glutamine Synthetase (GS) Activity in Tissue extracts, Cell lysate Samples.

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I. INTRODUCTION

Glutamine Synthetase (GS) is mainly present in plants, is one of the key enzymes in vivo assimilation of ammonia. GS can catalyze ammonium ions and glutamic acid to synthesise glutamine Gln, not only can prevent excessive biological toxic ammonium ions, but also glutamine Gln is the main storage and transport in the form of ammonia.

In the presence of ATP and Mg$^{2+}$, GS can catalyze ammonium ions and glutamic acid to synthesise glutamine Gln; glutamine Gln further converted to Gamma-glutamyl hydroxamic acid, under acidic conditions to form a red iron complexes; the complex has a maximum absorption peak at 540nm.
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>Reaction Buffer I</td>
<td>10 ml x 1</td>
<td>-20 °C</td>
</tr>
<tr>
<td>Reaction Buffer II</td>
<td>10 ml x 1</td>
<td>-20 °C</td>
</tr>
<tr>
<td>Substrate</td>
<td>Powder x 1</td>
<td>-20 °C</td>
</tr>
<tr>
<td>Dye Reagent</td>
<td>10 ml x 1</td>
<td>4 °C</td>
</tr>
</tbody>
</table>

**Note:**
Substrate: add 7 ml distilled water into the bottle to dissolve it absolutely before use, store at -20 °C.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer
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, intervention 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.

3. For liquid samples
Detect directly.
V. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Sample</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Buffer I</td>
<td>160 μl</td>
<td>--</td>
</tr>
<tr>
<td>Reaction Buffer II</td>
<td>--</td>
<td>160 μl</td>
</tr>
<tr>
<td>Substrate</td>
<td>70 μl</td>
<td>70 μl</td>
</tr>
<tr>
<td>Sample</td>
<td>70 μl</td>
<td>70 μl</td>
</tr>
</tbody>
</table>

Mix, put it in the oven, 37 °C for 30 minutes.

| Dye Reagent     | 100 μl | 100 μl |

Mix, centrifuged at 8000g, room temperature for 10 minutes, add 200 μl supernatant into the microplate, record absorbance measured at 540 nm.
VI. CALCULATION

**Unit Definition:** one unit is defined as the OD value changed 0.01 in the per ml/g reaction system per minute.

1. According to the protein concentration of sample

\[
GS \ (U/mg) = (OD_{Sample} - OD_{Control}) \times V_{Total} / (V_{Sample} \times C_{Protein}) / 0.01 / T
\]

\[
= 19 \times (OD_{Sample} - OD_{Control}) / C_{Protein}
\]

2. According to the weight of sample

\[
GS \ (U/g) = (OD_{Sample} - OD_{Control}) \times V_{Total} / (V_{Sample} \times W / V_{Assay}) / 0.01 / T
\]

\[
= 19 \times (OD_{Sample} - OD_{Control}) / W
\]

- \(C_{Protein}\): the protein concentration, mg/ml;
- \(W\): the weight of sample, g;
- \(V_{Total}\): the total volume of the enzymatic reaction, 0.4 ml;
- \(V_{Sample}\): the volume of sample, 0.07 ml;
- \(V_{Assay}\): the volume of Assay buffer, 1 ml;
- \(T\): the reaction time, 30 minutes.
VII. TECHNICAL SUPPORT

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

VIII. NOTES