Detection and Quantification of Beta-1,3-Glucanase Activity in Tissue extracts, Cell lysate Samples.

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

β-1,3-glucanase (EC 3.2.1.73) mainly exists in plant, and it catalyzes the hydrolysis of β-1,3-glucoside bond. Plant cells would induced to synthesize large amounts of β-1,3-glucanase when they are infected or in extreme environments. Thus, β-1,3-glucanase enzyme assays are widely applied in the research of plant pathology and adversity physiology.

β-1,3-glucanase could hydrolyse laminarin, and cut β-1,3-glucoside bond to produce reducing terminus. So generating rates of reducing sugar could calculate the activity of enzymes.
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>Dye Reagent</td>
<td>10 ml x 1</td>
<td>4 °C</td>
</tr>
<tr>
<td>Standard</td>
<td>Powder x 1</td>
<td>4 °C</td>
</tr>
<tr>
<td>Plate Adhesive Strips</td>
<td>3 Strips</td>
<td></td>
</tr>
</tbody>
</table>

**Note:**

**Substrate:** add 5 ml distilled water to dissolve before use.

**Standard:** add 1 ml distilled water to dissolve before use, mix; then add 0.3 ml into 0.7 ml distilled water, the concentration will be 3 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer
9. Convection oven
IV. SAMPLE PREPARATION

1. For tissue samples
Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 12000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For cell and bacteria samples
Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 12000g 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 into 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>50 μl</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Distilled water</td>
<td>--</td>
<td>50 μl</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Substrate</td>
<td>50 μl</td>
<td>50 μl</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Mix, put it in the oven, 37 °C for 10 minutes.

| Standard        | --     | --      | 100 μl   | --    |
| Distilled water | --     | --      | --       | 100 μl |
| Dye Reagent     | 100 μl | 100 μl  | 100 μl   | 100 μl |

Mix, put it into the convection oven, 90 °C for 10 minutes, record absorbance measured at 540nm.

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
VI. CALCULATION

**Unit Definition:** One unit of β-1,3-glucanase activity is defined as the enzyme liberates 1 μmol of reducing sugar per minute.

1. According to the protein concentration of sample

\[
\text{β-1,3-glucanase (U/mg)} = \frac{\text{C}_\text{Standard} \times \text{V}_\text{Standard}}{\text{C}_\text{Protein} \times \text{V}_\text{Sample}} \times \frac{\text{OD}_\text{Sample} - \text{OD}_\text{Control}}{\text{OD}_\text{Standard} - \text{OD}_\text{Blank}} \times \frac{\text{T}}{\text{C}_\text{Protein}}
\]

2. According to the weight of sample

\[
\text{β-1,3-glucanase (U/g)} = \frac{\text{C}_\text{Standard} \times \text{V}_\text{Standard}}{\text{C}_\text{Protein} \times \text{W} / \text{V}_\text{Assay}} \times \frac{\text{OD}_\text{Sample} - \text{OD}_\text{Control}}{\text{OD}_\text{Standard} - \text{OD}_\text{Blank}} \times \frac{\text{T}}{\text{W}}
\]

*C*\text{Standard}: the standard concentration, 3 mmol/L = 3 μmol/ml;

*C*\text{Protein}: the protein concentration, mg/ml;

*W*: the weight of sample, g;

*V*\text{Standard}: the volume of standard, 0.1 ml;

*V*\text{Sample}: the volume of sample, 0.05 ml;

*V*\text{Assay}: the volume of Assay buffer, 1 ml;

*T*: the reaction time, 10 minutes.
VII. TYPICAL DATA

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

![Standard curve graph]

Detection Range: 0.3 mmol/L - 3 mmol/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