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

\(\beta-1,3\)-glucanase (EC 3.2.1.73) mainly exists in plant, and it catalyzes the hydrolysis of \(\beta-1,3\)-glucoside bond. Plant cells would induced to synthesize large amounts of \(\beta-1,3\)-glucanase when they are infected or in extreme environments. Thus, \(\beta-1,3\)-glucanase enzyme assays are widely applied in the research of plant pathology and adversity physiology. \(\beta-1,3\)-glucanase could hydrolyse laminarin, and cut \(\beta-1,3\)-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 (500 µg/ml)</td>
<td>1 ml 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.

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 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 $5 \times 10^6$ cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 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>50 μl</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>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Mix, put it in the oven, 37 °C for 30 minutes. Then put it in boiling water for 10 minutes. Add the supernatant into the microplate.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Sample</th>
<th>Control</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant</td>
<td>100 μl</td>
<td>100 μl</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>--</td>
<td>--</td>
<td>100 μl</td>
<td>--</td>
</tr>
<tr>
<td>Distilled water</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>100 μl</td>
</tr>
<tr>
<td>Dye Reagent</td>
<td>100 μl</td>
<td>100 μl</td>
<td>100 μl</td>
<td>100 μl</td>
</tr>
</tbody>
</table>

Mix, put it in the oven, 95 °C for 10 minutes, record absorbance measured at 540nm.
VI. CALCULATION

**Unit Definition:** One unit of β-1,3-glucanase activity is the enzyme that generates 1 μg of reducing sugar per minute.

1. According to the protein concentration of sample

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

\[
= 33.33 \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})} / C_{\text{Protein}}
\]

2. According to the weight of sample

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

\[
= 33.33 \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})} / W
\]

*C_{\text{Standard}}: the protein concentration, 500 μg/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, 30 minutes.*
VII. TYPICAL DATA

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

![Graph showing a standard curve with the equation y = 0.0002x^{1.5693} and R^2 = 0.9993. The detection range is 50 μg/mL - 500 μg/mL.]

Detection Range: 50 μg/mL - 500 μg/mL

VIII. TECHNICAL SUPPORT

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

IX. NOTES