Phenylalanine ammonia-lyase
Microplate Assay Kit
User Manual

Catalog # CAK1018

Detection and Quantification of Phenylalanine ammonia-lyase (PAL) activity in Tissue extracts, Cell lysate Samples.

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

PAL widely found in various plants and a few micro-organisms, is a key enzyme in plants phenylpropanoid metabolism, and closely related to some important secondary substances synthetic such as lignin, isoflavones phytoalexin, flavonoid pigments, and play an important role in normal growth and development in plants and against the bacteria resist.

PAL catalytic cracking L-phenylalanine for trans-cinnamic acid and ammonia, trans-cinnamic acid has the maximum absorption value at 290 nm, PAL activity is calculated by measuring the absorbance increased rate.
II. KIT COMPONENTS

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-Well UV 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</td>
<td>30 ml x 1</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>4 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 10 ml Distilled water to dissolve before use, store at 4 °C.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 290 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 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 into the microplate:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Sample</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>10 μl</td>
<td>--</td>
</tr>
<tr>
<td>Reaction Buffer</td>
<td>120 μl</td>
<td>120 μl</td>
</tr>
<tr>
<td>Substrate</td>
<td>50 μl</td>
<td>50 μl</td>
</tr>
</tbody>
</table>

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

| Stop Solution    | 20 μl  | 20 μl   |
| Sample           | --     | 10 μl   |

Mix, record absorbance measured at 290nm immediately.
VI. CALCULATION

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

1. According to the protein concentration of sample

\[
\text{PAL (U/mg)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) \times V_{\text{Total}}}{(V_{\text{Sample}} \times C_{\text{Protein}}) \times 0.01 / T}
\]

\[
= 66.7 \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{C_{\text{Protein}}}
\]

2. According to the weight of sample

\[
\text{PAL (U/g)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) \times V_{\text{Total}}}{(V_{\text{Sample}} \times W / V_{\text{Assay}}) \times 0.01 / T}
\]

\[
= 66.7 \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{W}
\]

\(C_{\text{Protein}}\): the protein concentration, mg/ml;

\(W\): the weight of sample, g;

\(V_{\text{Total}}\): the total volume of the enzymatic reaction, 0.2 ml;

\(V_{\text{Sample}}\): the volume of sample, 0.01 ml;

\(V_{\text{Assay}}\): 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