Xanthine Oxidase Microplate Assay Kit
User Manual

Catalog # CAK1056

Detection and Quantification of Xanthine Oxidase (XOD) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

Xanthine Oxidase (XO) catalyzes the sequential oxidation of hypoxanthine to xanthine, and xanthine to uric acid and hydrogen peroxide. In humans and other primates, XO controls the final step of purine catabolism and is normally found in the liver and the intestinal mucosa. In rodents, XO is broadly expressed in most tissues. While XO activity is normally very low in blood, liver injury can result in the release of XO into blood. XO may contribute to the pathogenesis of gout and cardiovascular disease, and XO activity or expression may be upregulated in these conditions. Xanthine Oxidase Microplate Assay Kit is a sensitive assay for determining Xanthine Oxidase activity in various samples. The enzyme catalysed reaction products quinone can be measured at a colorimetric readout at 505 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>Diluent</td>
<td>15 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>Dye Reagent</td>
<td>Powder x 1</td>
<td>-20 °C, keep in dark</td>
</tr>
<tr>
<td>Standard (5 mmol/L)</td>
<td>1 ml x 1</td>
<td>4 °C, keep in dark</td>
</tr>
<tr>
<td>Plate Adhesive Strips</td>
<td>3 Strips</td>
<td></td>
</tr>
</tbody>
</table>

Note:

Substrate: add 5 ml Diluent to dissolve before use.

Dye Reagent: add 10 ml Diluent to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 505 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 20 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 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples
Detect directly.
V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Standard</th>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>Dye Reagent</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>Standard</td>
<td>50 µl</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Distilled water</td>
<td>--</td>
<td>50 µl</td>
<td>--</td>
</tr>
<tr>
<td>Sample</td>
<td>--</td>
<td>--</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

Mix, put it in the oven, 37 °C for 5 minutes, measured at 505 nm and record the absorbance.
VI.  CALCULATION

Unit Definition: One unit of XOD activity is defined as the enzyme generates 1 µmol H2O2 per minute.

1. According to the protein concentration of sample
   \[
   \text{XOD (U/mg)} = \frac{\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})} \times \frac{\text{V}_{\text{Sample}} \times \text{C}_{\text{Protein}}}{\text{T}}
   \]
   \[
   = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})} \times \frac{\text{V}_{\text{Sample}}}{\text{C}_{\text{Protein}}} / \text{T}
   \]

2. According to the weight of sample
   \[
   \text{XOD (U/g)} = \frac{\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})} \times \left(\frac{\text{W} \times \text{V}_{\text{Sample}}}{\text{V}_{\text{Assay}}}\right) / \text{T}
   \]
   \[
   = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})} \times \frac{\text{W}}{\text{V}_{\text{Assay}}} / \text{T}
   \]

3. According to the quantity of cells or bacteria
   \[
   \text{XOD (U/10}^4) = \frac{\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})} \times \left(\frac{\text{N} \times \text{V}_{\text{Sample}}}{\text{V}_{\text{Assay}}}\right) / \text{T}
   \]
   \[
   = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})} \times \frac{\text{N}}{\text{V}_{\text{Assay}}} / \text{T}
   \]

4. According to the volume of serum or plasma
   \[
   \text{XOD (U/ml)} = \frac{\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})} \times \frac{\text{V}_{\text{Sample}}}{\text{T}}
   \]
   \[
   = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})}
   \]

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

**W**: the weight of sample, g;

**N**: the quantity of cell or bacteria, N \times 10^4;

**C_{\text{Standard}}**: the standard concentration, 10 mmol/L = 10 µmol/ml;

**V_{\text{Standard}}**: the volume of the standard, 0.05 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, 5 minutes.

VII. TYPICAL DATA

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

![Graph showing a linear relationship between OD 565 nm and Standard curve (mmol/L). The equation is y = 0.183x + 0.025, and R^2 = 0.9987.]

Detection Range: 0.05 mmol/L - 5 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