Supplementary File S3.
Methodology of Catalase (CAT) Assay

**CATALASE ASSAY**

**Colorimetric Method**

**For Research Use Only**

**PROCEDURE**:

Dilute R2 1000 times immediately before use. (10 uL + 10 ml d. Water). Discard after use.

Dilute Sample if necessary.

<table>
<thead>
<tr>
<th>Sample Blank (ml)</th>
<th>Sample (ml)</th>
<th>Standard Blank (ml)</th>
<th>Standard (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>0.05</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>D. H2O</td>
<td>0.05</td>
<td>-</td>
<td>0.10</td>
</tr>
<tr>
<td>R1</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>R2</td>
<td>-</td>
<td>0.10</td>
<td>-</td>
</tr>
</tbody>
</table>

Incubate exactly ONE min. at 25°C then add:

| R3                | 0.20        | 0.20                | 0.20          | 0.20          |
| R4                | 0.50        | 0.50                | 0.50          | 0.50          |

Incubate 10 min. at 37°C, read sample (A_{Sample}) against sample blank and standard (A_{Standard}) against Standard blank at 510 nm (500 – 520 nm). Color stable for one hour.

**CALCULATION**:

**Catalase Activity**:

In Plasma (U/L) =

\[
\frac{A_{\text{standard}} - A_{\text{Sample}}}{A_{\text{standard}}} \times 1000
\]

In Tissue (U/g) =

\[
\frac{A_{\text{standard}} - A_{\text{Sample}}}{A_{\text{standard}}} \times \frac{1}{\text{gm tissue used per test}}
\]

**REFERENCE**:

SAMPLE PREPARATION

**Tissue Homogenate**

1. Prior to dissection, perfuse tissue with a PBS (phosphate buffered saline) solution, pH 7.4, containing 0.16 mg/ml heparin to remove any red blood cells and clots.
2. Homogenize the tissue in 5–10 ml cold buffer (i.e., 50 mM potassium phosphate, pH 7.4, 1 mM EDTA and 1 mL/L Triton X-100) per gram tissue.
3. Centrifuge at 4,000 rpm for 15 minutes at 4°C.
4. Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.

**Plasma**

1. Collect blood using an anticoagulant such as heparin, citrate, or EDTA.
2. Centrifuge at 4,000 rpm for 15 minutes at 4°C.
3. Collect the plasma for assaying and store on ice. If not assaying on the same day, freeze at -80°C. The sample will be stable for at least one month.

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**CATALASE ASSAY**

- **Colorimetric Method**
  - +4 to +8°C
  - 25 Tests

**REAGENTS**

- **R1** Buffer 25 ml
- **R2** H$_2$O$_2$ 2 ml
- **R3** Chromogen-Inhibitor 10 ml
- **R4** Enzyme 25 ml

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