DESCRIPTION

DIAMINE OXIDASE (DAO) also known as histaminase or amine oxidase (copper containing), is an enzyme involved in the metabolism, oxidation, and inactivation of histamine in animals. Highest content is observed in the digestive tract and placenta. An imbalance between histamine intake and the capacity for histamine degradation can lead to histamine intolerance (HIT). Measuring DAO activity in serum can be useful in diagnosing HIT.

BioAssay Systems’ non-radioactive, fluorimetric DAO assay is based on the oxidation of putrescine to pyrroline plus NH₂ and H₂O₂. The generated H₂O₂ is then used by HRP to oxidize a dye making it fluorescent. The increase in fluorescence at λex/em = 530/585 nm is directly proportional to the enzyme activity.

KEY FEATURES

Fast and sensitive. Use of 10 µL sample. Linear detection range 0.5 to 6 U/L for 30 min reaction at 25°C.

Convenient. The procedure involves adding a single working reagent, and reading the fluorescence at 0 and 30 minutes. Room temperature assay. No 37°C heater is needed.

High-throughput. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

APPLICATIONS

Direct Assays: DAO activity in serum or plasma samples.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>10 mL</td>
</tr>
<tr>
<td>HRP Enzyme</td>
<td>120 µL</td>
</tr>
<tr>
<td>Substrate</td>
<td>120 µL</td>
</tr>
<tr>
<td>H₂O₂ Standard</td>
<td>100 µL</td>
</tr>
<tr>
<td>Dye Reagent</td>
<td>120 µL</td>
</tr>
</tbody>
</table>

Storage conditions. The kit is shipped at ambient temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Procedure using 96-well plate

1. **Internal Standard.** First prepare 500 µL of 8.82 mM H₂O₂ by mixing 5 µL of the H₂O₂ Standard (882 mM) and 495 µL dH₂O. Next mix 20 µL of the 8.82 mM H₂O₂ with 960 µL dH₂O to make a 180 µM internal standard. Use diluted H₂O₂ within 1 hour.

2. **Prepare sufficient Working Reagent (WR) for all Sample wells by mixing, for each well: 85 µL Assay Buffer, 1 µL HRP Enzyme, 1 µL Substrate and 1 µL Dye Reagent.** Prepare sufficient Blank Working Reagent (BWR) for all Sample Blank and Internal Standard wells by mixing for each well: 85 µL Assay Buffer, 1 µL HRP Enzyme, 1 µL Dye Reagent (i.e. no Substrate).

3. Transfer 10 µL of each sample into three separate wells of a black, flat-bottom 96-well plate: one well for Sample measurement (Fₘₙₐₜ), one for Sample Blank (Fₘₙₐₚ) and one for the Internal Standard (Fₘₙₐₛₚ).

4. Transfer 10 µL dH₂O to the Sample and Sample Blank wells. Transfer 10 µL of the 180 µM H₂O₂ to the Internal Standard wells.

5. Transfer 80 µL WR to each Sample well. Transfer 80 µL BWR to each Sample Blank and Internal Standard well.

6. Read fluorescence at λex/em = 530/585 nm at time 0 and again at time 30 min.

**CALCULATION**

Subtract the time 0 fluorescence from the time 30 fluorescence for the Sample and Sample Blank wells to compute ∆FS and ∆FSB respectively.

The DAO activity can then be computed as follows:

\[
\text{DAO Activity} = \frac{\Delta F_S - \Delta F_{SB}}{F_{SB30} - F_{SB30}} \times \frac{180 \mu M}{t (min)} \times n \ (U/L)
\]

where FSB30 and FSB30 are the fluorescence readings taken at 30 min for the Internal Standard and Sample Blank respectively and t is the reaction time (30 minutes). n is the sample dilution factor.

**LITERATURE**