Technical Bulletin

Diamine Oxidase Assay Kit

Catalogue number MAK526

Product 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 levels are 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.

The Diamine Oxidase Assay Kit is a non-radioactive, fluorometric assay based on the oxidation of putrescine to pyrroline plus NH_3 and H_2O_2. The generated H_2O_2 is then used by HRP to oxidize a dye making it fluorescent. The increase in fluorescence at λ_{Ex} =530 nm/λ_{Em} =585 nm is directly proportional to the enzyme activity.

The linear detection range of the kit 0.5 to 6 U/L for a 30-minute reaction at 25 °C. The kit is suitable for the determination of DAO activity in serum or plasma samples.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK526A
- HRP Enzyme 120 µL
  Catalogue Number MAK526B
- Substrate 120 µL
  Catalogue Number MAK526C
- Dye Reagent 120 µL
  Catalogue Number MAK526D
- H_2O_2 Standard (882 mM) 100 µL
  Catalogue Number MAK526E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Fluorescent multiwell plate reader
- Black flat-bottom 96-well or 384-well plates. Cell culture or tissue culture treated plates are not recommended.

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Internal Standard

1. Prepare 8.82mM H_2O_2: Prepare 500 µL of 8.82 mM H_2O_2 by mixing 5 µL of the H_2O_2 Standard (882 mM) with 495 µL purified water.
2. Prepare 180 µM internal Standard: Mix 20 µL of the 8.82 mM H_2O_2 with 960 µL purified water to make a 180 µM internal Standard.

Note: Use diluted H_2O_2 within 1 hour

Working Reagent

Prepare Working Reagent as per Table 1. Each well requires 80 µL of Working Reagent. Prepare enough for the assay.

Table 1.
Preparation of Working Reagent.

Reagent	Volume (µL)
Assay Buffer	85
HRP Enzyme	1
Substrate	1
Dye Reagent	1

Blank Working Reagent

Prepare Blank Working Reagent as per Table 2. Each well requires 80 µL of Blank Working Reagent. Prepare enough for the assay.

Table 1.

Preparation of Blank Working Reagent.

Reagent	Volume (µL)
Assay Buffer	85
HRP Enzyme	1
Dye Reagent	1

Standard Curve Preparation

1. Transfer 10 µL of each Sample into three separate wells of a black, flatbottom 96-well plate.
2. Add 10 µL purified water to the Sample and blank wells.
3. Add 10 µL of the 180 µM H_2O_2 to the Standard wells.
4. Transfer 80 µL Working Reagent to each Sample well.
5. Transfer 80 µL Blank Working Reagent to each Sample Blank and Internal Standard well.

Measurement

Read fluorescence:
(F) at λ_{Ex} = 530 nm/ λ_{Em} = 585 nm at time 0 and again at time 30 minutes.

Results

1. Subtract the time 0 fluorescence from the time 30 fluorescence for the Sample and Sample Blank wells to compute ΔF_S and ΔF_{SB} respectively.
2. The DAO activity can then be computed as follows:
   DAO Activity (U/L) =

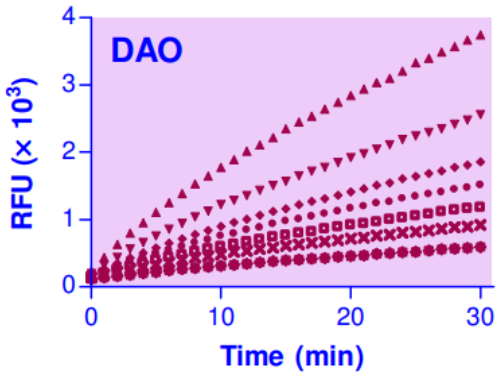
$$\frac{\Delta F_S - \Delta F_{SB}}{F_{IS30} - F_{SB30}} \times \frac{180\mu M}{t\ (min)} \times DF$$

Where:
ΔF_S = Fluorescence intensity of the Sample well
ΔF_{SB} = Fluorescence intensity of the Sample Blank well
F_{IS30} = fluorescence readings taken at 30 min for the Internal Standard
F_{SB30} = fluorescence readings taken at 30 min for the Sample Blank
t = Reaction Time (30 minutes)
DF = Sample dilution factor

Note: If the sample activity is higher than 6 U/L, dilute sample in water and repeat the assay. Multiply the results by the dilution factor. Alternatively, the reaction can be run for a shorter length of time.

Unit definition: 1 Unit (U) of DAO will catalyze the conversion of 1 µmole of putrescine to pyrroline plus NH3 and H_2O_2 per min at pH 7.5.

Figure 1.
Typical curve for DAO Titration in Human Serum.



DAO Titration in Human Serum

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