Diamine Oxidase (DAO) Assay Kit (Fluorometric)

LS-K305-100 (100 Tests) • **Store at -20°C**

Introduction

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. This non-radioactive, fluorometric DAO assay is 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 $\lambda_{ex/em}$ = 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. "Add-mix-read" type assay. 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.

Components

Component	K305-100
	100 Tests
Assay Buffer	10 mL
Substrate	120 µL
Dye Reagent	120 µL
HRP Enzyme	120 µL
H_2O_2 Standard	100 µL

Materials Not Supplied

Pipetting devices, centrifuge tubes, black flat-bottom 96-well plates (e.g. Greiner cat# 655209), and plate reader.

Storage

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

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Assay Procedure

Procedure Using 96-Well Plate

1. Internal Standard. First prepare 500 µL of 8.82 mM H_2O_2 by mixing 5 µL of the H_2O_2 Standard (882 mM) and 495 µL dH_2O. Next mix 20 µL of the 8.82 mM H_2O_2 with 960 µL dH_2O to make a 180 µM internal standard. Use diluted H_2O_2 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, and 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 (FS), one for Sample Blank (FSB) and one for the Internal Standard (FIS).

   Transfer 10 µL dH_2O to the Sample and Sample Blank wells. Transfer 10 µL of the 180 µM H_2O_2 to the Internal Standard wells.
4. Transfer 80 µL WR to each Sample well. Transfer 80 µL BWR to each Sample Blank and Internal Standard well.
5. Read fluorescence at $\lambda_{ex/em}$ = 530/585 nm at time 0 and again at time 30 min.

Calculations

Subtract the time 0 fluorescence from the time 30 fluorescence for the Sample and Sample Blank wells to compute ΔFS and ΔFSB respectively. The DAO activity can then be computed as follows:

$$\text{DAO Activity} = \frac{\Delta F_S - \Delta F_{SB}}{F_{IS30} - F_{SB30}} \times \frac{180\ \mu M}{t\ (\text{min})} \times n \quad (U/L)$$

where F_{IS30} and F_{SB30} 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.

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 NH_3 and H_2O_2 per min at pH 7.5.

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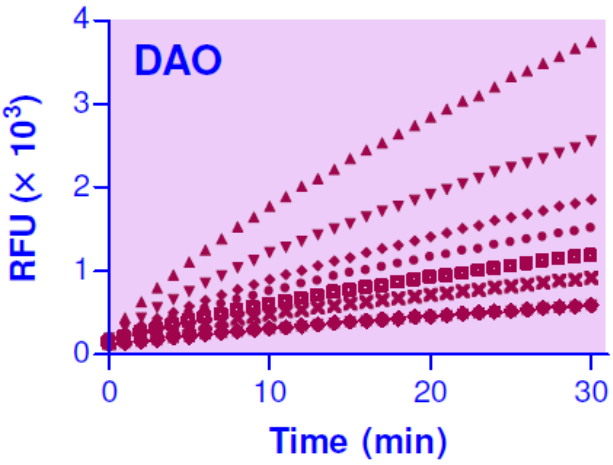
Sample Data



DAO Titration in Human Serum

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