

EZMeta™ Diamine Oxidase (DAO) Colorimetric Activity Kit

Cat #: D-AKC1220

Size: 48T / 96T

Storage: Stored at 4°C for 6 months, protected from light

Product Information

Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant, Bacteria, Urine

Assay Principle

DAO (EC1.4.3.6) is widely found in animals (intestinal mucosa, lung, liver, kidney, etc.), plants and microorganisms. It catalyzes the oxidation of diamines to aldehydes. Its activity is closely related to nucleic acid and protein synthesis, and can reflect the integrity and injury degree of intestinal mechanical barrier. EZMeta™ Diamine Oxidase (DAO) Colorimetric Activity Kit provides a convenient tool for detection of DAO Activity. The principle is that DAO catalyzes 1,5-pentanediamine to produce aldehyde and hydrogen peroxide.

The oxidation of o-Dianisidine(3,3'-Dimethoxybenzidine) with hydrogen peroxide to produce colored substances was catalyzed by exogenous Horseradish peroxidase, the colored substances have a maximum absorption peak detected at about 460 nm. The enzyme activity of DAO was calculated by detecting the rate of increase in absorption at 460 nm. The kit can detect serum (plasma), animal and plant tissues, cells, bacteria, cell supernatants, urine and other samples

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60 mL×2	4°C
Reagent I	0.2 mL	0.4 mL	4°C, protected from light
Reagent II	1	1	4°C, protected from light
Reagent III	1	1	4°C, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 460 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, ice maker, incubator
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent I : Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Reagent II Solution: Add 4 mL deionized water for 96 T or 2 mL deionized water for 48 T to dissolve before use. This solution can be stored at 4°C for 1 week. The solution can also be stored at -20°C from light after aliquoting to avoid repeated freezing and thawing.

Reagent III Solution: Add 2 mL deionized water for 96 T or 1 mL deionized water for 48 T to dissolve before use. This solution can be stored at -20°C from light after aliquoting to avoid repeated freezing and thawing.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80°C for one month.

1. Animal Tissues: Weigh 0.1 g tissues, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
2. Plant Tissues: Weigh 0.1 g tissues, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
3. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
4. Serum, Plasma, Cell Supernatant, Urine or other liquid samples: Tested directly.

Note: It will be better to quantify the total protein with EZMeta™ Diamine Oxidase (DAO) Colorimetric Activity Kit, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 460 nm, visible spectrophotometer was returned to zero with deionized water.
2. Preheat the incubator to 37°C.
3. Add the following reagents respectively into the 96-well microplate or microglass cuvette:

Reagent	Control Well (μL)	Test Well (μL)
Sample	50	50
Extraction Buffer	128	108
Reagent I	2	2
Reagent II Solution	20	20
Reagent III Solution	0	20

Mix well, incubate in 37°C for 5 min. Then reading the values at 460 nm. Finally, calculate $\Delta A = A_{\text{Test}} - A_{\text{Control}}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.005, increase the sample quantity appropriately. If ΔA is greater than 0.8, the sample can be appropriately diluted, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well plates calculation formula

1. Calculation the activity of DAO in animal and plant tissues

(1) Calculated by protein concentration

Unit definition: 1 μmol Oxidized o-Dianisidine produced per min in 1 mg tissues protein reaction system is defined as a unit of enzyme activity.

$$\text{DAO (U/mg prot)} = \Delta A \div d \div \epsilon \times V_{\text{Reaction Total}} \div (\text{Cpr} \times V_{\text{Sample}}) \div T \times n = \mathbf{213 \times \Delta A \div \text{Cpr} \times n}$$

(2) Calculated by fresh weight of samples

Unit definition: 1 μmol Oxidized o-Dianisidine produced per min in 1 g tissues reaction system is defined as a unit of enzyme activity.

$$\text{DAO (U/g)} = \Delta A \div d \div \epsilon \times V_{\text{Reaction Total}} \div (W \div V_{\text{Extraction}} \times V_{\text{Sample}}) \div T \times n = \mathbf{213 \times \Delta A \div W \times n}$$

2. Calculate the activity of DAO in liquid sample

Unit definition: 1 μmol Oxidized o-Dianisidine produced per min in 1mL liquid sample reaction system is defined as a unit of enzyme activity.

$$\text{DAO (U/mL)} = \Delta A \div d \div \epsilon \times V_{\text{Reaction Total}} \div V_{\text{Sample}} \div T \times n = 213 \times \Delta A \times n$$

3. Calculated the activity of DAO by cells or bacteria number

Unit definition: 1 μmol Oxidized o-Dianisidine produced per min in 10^4 cells or bacteria reaction system is defined as a unit of enzyme activity.

$$\text{DAO (U/10}^4\text{)} = \Delta A \div d \div \epsilon \times V_{\text{Reaction Total}} \div (500 \times V_{\text{Sample}} \div V_{\text{Extraction}}) \div T \times n = 0.427 \times \Delta A \times n$$

Where: $\Delta A = A_{\text{Test}} - A_{\text{Control}}$; d: 96-well plate diameter, 0.5 cm; ϵ : Oxidized o-Dianisidine molar extinction coefficient, $7.5 \times 10^{-3} \text{ mL}/\mu\text{mol}/\text{cm}$; $V_{\text{Reaction Total}}$: total reaction volume, 0.2 mL; Cpr: sample protein concentration, mg/mL; V_{Sample} : sample volume added, 0.05 mL; T: reaction time, 5 min; n: dilution factor; W: sample weight, g; $V_{\text{Extraction}}$: Extraction Buffer volume added, 1 mL; 500: Total number of bacteria or cells, 5×10^6 .

B. Microglass cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

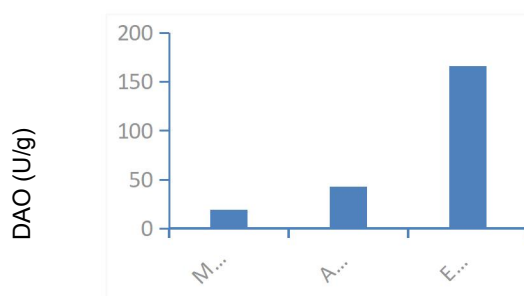


Figure1. DAO activity in mouse spleen, aloe and epipremnum aureum respectively. Assays were performed following kit protocol

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.