Diamine Oxidase(DAO) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC1285

Sizes：100T/48S

Components:

Extract solution: 70 mL×1. Storage at 4℃;

Reagent I: 0.25 mL×1. Storage at 4℃;

Reagent II: Powder×1. Add 2 mL of distilled water when the solution will be used. The reagent can be stored at 4℃ for a month.

Reagent III: 2 mL×1. Storage at 4℃.

Product Description：

Diamine oxidase (DAO, EC1.4.3.6) widely exists in animals (intestinal mucosa, lung, liver, kidney, etc.), plants and microorganisms. It can catalyze the oxidation of polyamines to aldehydes, and its activity is closely related to the synthesis of nucleic acids and proteins. It can reflect the integrity and damage degree of intestinal mechanical barrier.

DAO catalyzes the production of aldehydes and hydrogen peroxide from cadaverine, and it can catalyze the oxidation of o-dianisidine by hydrogen peroxide to generate colored substances with adding excessive horseradish peroxidase. This colored substance has a characteristic absorption at 500 nm. The activity of DAO can be calculated by measuring the absorbance increase rate of this wavelength.

Reagents and Equipment Required but Not Provided：

Spectrophotometer/Microplate reader, low temperature centrifuge, 96-well flat-bottom plates/glass cuvette, scales, distilled water.

Procedure

I. Sample preparation:

1. Tissue

The mass of tissue (g): the volume of Extract solution(mL) is 1:5~10(it is suggested to take about 0.1 g of tissue and add 1 mL of Extract solution). fully grinding on ice. Centrifuge at 10000 ×g for 20 minutes at 4℃, take the supernatant and place it on ice for test.

2. Bacteria or cells

The number of bacteria or cells (10^4): the volume of Extract solution (mL) is 500~1000:1(it is suggested to take about 5 million bacteria/cell and add 1 mL of Extract solution). Bacteria and cells is split by ultrasonic (Power: 300 W, work time 3s, interval 7s, total time: 3 minutes). Centrifuge at 10000 ×g for 10 minutes at 4℃, take the supernatant and place it on ice for test.

3. Serum (plasma) sample: Detect sample directly.

II. Determination procedure：

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 500 nm, set zero with distilled water.
2. Add reagents with the following list:

Name of reagent(μL)	Control tube(A_C)	Test tube(A_T)
Crude enzyme solution	50	50
Extract solution	108	108
Reagent I	2	2
Reagent II	20	20
Reagent III	-	10

Mix well. After 30 minutes of water bath at 37°C, measure the absorbance value at 500 nm. $\Delta A = A_T - A_C$.

III. Calculations：

A. The calculation formula of 96-well plate

1. Tissue

a. Protein concentration

Unit definition: one unit is defined as the amount of enzyme catalyze the produce of 1 μmol oxidized o-dianisidine per minute in reaction volume every milligram of tissue protein.

$DAO(U/mg\ prot) = \Delta A \div d \div \varepsilon \times V_{rv} \div (C_{pr} \times V_{sv}) \div T = 30 \times \Delta A \div C_{pr}$

b. Sample weight

Unit definition: one unit is defined as the amount of enzyme catalyze the produce of 1 μmol oxidized o-dianisidine per minute in reaction volume every gram of tissue.

$DAO(U/g\ fresh\ weight) = \Delta A \div d \div \varepsilon \times V_{rv} \div (W \div V_s \times V_{sv}) \div T = 30 \times \Delta A \div W$

2. Serum (plasma) sample

Unit definition: one unit is defined as the amount of enzyme catalyze the produce of 1 μmol oxidized o-dianisidine per minute in reaction volume every milliliter serum(plasma).

$DAO(U/mL) = \Delta A \div d \div \varepsilon \times V_{rv} \div V_{sv} \div T = 30 \times \Delta A$

3. Cell amount

Unit definition: one unit is defined as the amount of enzyme catalyze the produce of 1 μmol oxidized o-dianisidine per minute in reaction volume every 10 thousand bacteria or cells.

$POD(U/10^4\ cell) = \Delta A \div d \div \varepsilon \times V_{rv} \div (500 \div V_s \times V_{sv}) \div T = 0.06 \times \Delta A$

V_{rv}: Total reaction volume, 0.2 mL;

V_{sv}: Volume of crude enzyme, 0.05 mL;

V_s: Extract solution volume, 1 mL;

C_{pr}: Sample protein concentration, mg/mL;

W: Sample weight, g;

d: Light path, 0.6 cm;

ε: Extinction coefficient of oxidized o-dianisidine, 7.5×10^{-3} mL/μmol/cm;

T: Reaction time, 30 minutes.

B. The calculation formula of cuvette

1. Tissue

a. Protein concentration

Unit definition: one unit is defined as the amount of enzyme catalyze the produce of 1 μmol of oxidized o-dianisidine per minute in reaction volume every milligram of tissue protein.

$DAO(U/mg\ prot) = \Delta A \div d \div \varepsilon \times Vrv \div (Cpr \times Vsv) \div T = 18 \times \Delta A \div Cpr$

b. Sample weight

Unit definition: one unit is defined as the amount of enzyme catalyze the produce of 1 μmol of oxidized o-dianisidine per minute in reaction volume every gram of tissue.

$DAO(U/g\ fresh\ weight) = \Delta A \div d \div \varepsilon \times Vrv \div (W \div Vs \times Vsv) \div T = 18 \times \Delta A \div W$

2. Serum (plasma) sample

Unit definition: one unit is defined as the amount of enzyme catalyze the produce of 1 μmol of oxidized o-dianisidine per minute in reaction volume every milliliter of serum(plasma).

$DAO(U/mL) = \Delta A \div d \div \varepsilon \times Vrv \div Vsv \div T = 18 \times \Delta A$

3. Cell amount

Unit definition: one unit is defined as the amount of enzyme catalyze the produce of 1 μmol of oxidized o-dianisidine per minute in reaction volume every 10 thousand bacteria or cells.

$POD(U/10^4\ cell) = \Delta A \div d \div \varepsilon \times Vrv \div (500 \div Vs \times Vsv) \div T = 0.036 \times \Delta A$

Vrv: Total reaction volume, 0.2 mL;

Vsv: Volume of crude enzyme, 0.05 mL;

Vs: Extract solution volume, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

d: Light path, 1 cm;

ε: Extinction coefficient of oxidized o-dianisidine, 7.5×10^{-3} mL/μmol/cm;

T: Reaction time, 30 minutes.

Note:

1. If the OD value is less than 0.01, appropriately increase the quality of samples for extraction. If the OD value is greater than 0.8, the crude enzyme solution can be appropriately diluted, or reduce the quality of samples for extraction.

2. The protein content of the sample needs to be determined separately.

Examples:

1. Add 0.1g liver to 1mL extract solution and grind thoroughly on ice, centrifuge with 10000g at 4℃ for 20min, take supernatant on ice, follow the determination procedure to operate, with 96-well flat-bottom plates to calculate ΔA =A(T)-A(B)=0.135-0.125=0.01, according with mass of sample to calculate enzyme activity: DAO（U/g mass）=30×ΔA÷W=3 U/g mass.

2. Add 0.1g holly(Ilex chinensis Sims) to 1mL extract solution and grind thoroughly on ice, centrifuge with 10000g at 4℃ for 20min, take supernatant on ice, follow the determination procedure to

operate, with 96-well flat-bottom plates to calculate ΔA =A(T)-A(B)=0.139-0.126=0.013, according with mass of sample to calculate enzyme activity: DAO（U/g mass）=30×ΔA÷W=3.9 U/g mass.

Related Products:

BC0020/BC0025   Malondialdehyde (MDA) Content Assay Kit

BC1090/BC1095   Xanthine Oxidase(XOD) Activity Assay Kit

BC0690/BC0695   Glucose Oxidase (GOD) Activity Assay Kit

BC1270/BC1275   Protein Carbonyl Content Assay Kit