SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Diamine Oxidase(DAO) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: AK0458

Sizes： 50T/24S

Components:

Extract solution: 60 mL×1. Storage at 4℃;

Reagent I: 0.6 mL×1. Storage at 4℃;

Reagent II: Powder×1. Add 6 mL of distilled water when the solution will be used. The reagents can be stored at 4℃ for a month.

Reagent III: 1.5 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, low temperature centrifuge, 1 mL glass cuvette, water bath, 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 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 is 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 (mL)	Control tube (A_C)	Test tube (A_T)
Crude enzyme solution	0.25	0.25
Extract solution	0.59	0.59
Reagent I	0.01	0.01
Reagent II	0.1	0.1
Reagent III	-	0.05

Mix well. After 30 minutes of water bath at 37℃, measure the absorbance in 1 mL glass cuvette at 500 nm. $\Delta A = A_T - A_C$.

III. Calculations：

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) ＝ΔA÷(ε×d)×Vrv÷(Cpr×Vsv)÷T=18×ΔA÷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) ＝ΔA÷(ε×d)×Vrv÷(W÷Vs×Vsv)÷T=18×ΔA÷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)=ΔA÷(ε×d)×Vrv÷Vsv÷T=18×ΔA

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) ＝ΔA÷(ε×d)×Vrv÷(500÷Vs×Vsv)÷T=0.036×ΔA

Vrv: Total reaction volume, 1 mL;

Vsv: Volume of crude enzyme, 0.25 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;

500: Total number of bacteria or cells, 5 million.

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, and calculate ΔA =A(T)-A(B)=0.235-0.223=0.012, according with mass of sample to calculate enzyme activity: DAO（U/g mass）=18×ΔA÷W=2. 16 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, and calculate ΔA =A(T)-A(B)=0.215-0. 198=0.017, according with mass of sample to calculate enzyme activity: DAO（U/g mass）=18×ΔA÷W=3.06 U/g mass.

3. Take 0. 1g goose serum, follow the determination procedure to operate, and calculate ΔA =A(T)-A(B)=0.098-0.087=0.011, to calculate enzyme activity: DAO（U/g mass）=18×ΔA=0. 198U/mL.

Related Products:

AK0662/AK0612	Malondialdehyde (MDA) Content Assay Kit
AK0490/AK0489	Xanthine Oxidase(XOD) Activity Assay Kit
AK0522/AK0521	Glucose Oxidase (GOD) Activity Assay Kit
AK0460/AK0459	Protein Carbonyl Content Assay Kit