Diamine Oxidase (DAO) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/ Microplate reader

Catalog Number: BC1285

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Liquid 2 mL×1	2-8℃
Reagent II	Liquid 25 mL×1	2-8℃
Reagent III	Liquid 5 mL×1	2-8℃
Standard	Liquid 140 μL×1	2-8℃

Solution Preparation:

1. Standard goods: Liquid 102 μL×1. Store at 2-8℃. 1mol/L (1000μmol/mL) hydrogen peroxide solution was obtained by adding 898μL distilled water before use and stored at 2-8℃ for 4 weeks.
2. Preparation of 0.25μmol/mL standard solution: 10μL 1000μmol/mL standard solution and 990μL distilled water were mixed to form 10μmol/mL standard solution; Then 25μL 10μmol/mL standard solution and 975μL distilled water were mixed to form 0.25μmol/mL standard solution for use.

Product Description

DAO (EC1.4.3.6) is widely found in animals (intestinal mucosa, lung, liver, kidney, etc.), plants, and microorganisms. Catalytic oxidation of polyamines to aldehydes, whose activity is closely related to nucleic acid and protein synthesis, can reflect the integrity and damage degree of the intestinal mechanical barrier.

DAO catalyzed cadaverine to produce aldehydes and hydrogen peroxide, and Fe^{2+} in potassium ferricyanide oxidized by H_2O_2 to produce Fe^{3+}. Fe^{3+} reacted with 4-amino-antipyrine and phenol to produce red quinones with characteristic absorption peaks at 505nm. The activity of DAO was calculated by measuring the absorbance value at 505nm.

Reagents and Equipment Required but Not Provided.

Spectrophotometer/Microplate reader, mortar/homogenizer/cell ultrasonic crusher, centrifuge, constant temperature foster box/water-bath, centrifuge, micro quartz cuvette/96 well flat-bottom plate, ice and distilled water.

Procedure:

I. Sample preparation

1. **Bacteria or cells:** Collect bacteria or cells to centrifuge tube, and discard supernatant after

centrifuging. Add 1 mL of Extract solution to 5 million of bacteria or cells and use ultrasonic breaking bacteria or cells. (place on ice, ultrasonic power 300W, working time 3s, interval 7s, Total time: 3

minutes). Centrifuge at 10000 ×g for 20 minutes at 4℃ to remove insoluble materials and take the supernatant on ice for testing.

2. **Tissue:** Add 1 mL of Extract solution to 0.1 g of tissue, and homogenate on ice. Centrifuge at 10000 ×g for 20 minutes at 4℃ to remove insoluble materials and take the supernatant on ice for testing.
3. **Serum (plasma) sample:** detect sample directly. If the solution is turbidity, the supernatant is taken after centrifugation for determination.

II. Determination

1. Preheat the spectrophotometer or microplate reader for more than 30 minutes, adjust the wavelength to 505 nm and set spectrophotometer counter to zero with distilled water.
2. Sample Test (add Reagent in the EP tube/96 well flat-bottom plate):

Reagent（μL）	Test tube (T)	Control tube(C)	Standard tube (S)	Blank tube (B)
Sample	75	75	-	-
Standard goods	-	-	75	-
Reagent I	30	-	-	-
Reagent II	200	200	200	200
Reagent III	40	40	40	40
distilled water	-	30	30	105

Vortex mixing, 37℃ water bath or constant temperature incubator accurate reaction 2h. 10000g was centrifuged at 4℃ for 5min, and 200μL supernatant was put into microglass colorimetric dish / 96-well plate. The absorption value A at 505nm was determined and denoted as A_T, A_C, A_S and A_B. Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. Each measuring tube needs to set up a pair, standard curve and blank tube only need to be measured 1-2 times.

III. Calculation of DAO activity:

1. Drawing of standard curve:

According to the concentration of the standard tube (x, μmol/mL) and the absorbance ΔA_S (y, ΔA_S), the standard curve was established. According to the standard curve, the ΔA_T (y, ΔA_T) was substituted into the formula to calculate the sample concentration (x, μmol/mL).

2. Calculation of DAO activity:

1) Protein concentration:

Definition of unit: 1μmol H_2O_2 per mg of histamine decomposition per hour is defined as a unit of enzyme activity.

DAO activity（U/mg prot）= $x \times V_S \div (V_S \times Cpr) \div T \times F = 0.5x \div Cpr \times F$

2) Sample weight:
Definition of unit: 1μmol of H_2O_2 per g of tissue decomposition of cadaverine per hour is defined as a unit of enzyme activity.
DAO activity（U/g weight）= x ×V_S÷(W÷V_{EV}×V_S) ÷T×F=0.5x÷ W×F

3) Cell amount:
Definition of unit: 1μmol of H_2O_2 produced by decomposition of cadaverine per 104 cells per hour is defined as a unit of enzyme activity.
DAO activity（U/10^4 cell）= x ×V_S÷(N÷V_{EV}×V_S) ÷T×F=0.5x÷ N×F

4) Volume of liquid:
Definition of Unit: The production of 1μmol of H_2O_2 per mL of liquid decomposition of cademine per hour is defined as a unit of enzyme activity.
DAO activity（U/mL）= x ×V_S÷V_S ÷T×F=0.5x×F

V_S: Sample volume,0.075 mL.
V_{EV}: Extraction volume I, 1 mL;
Cpr: Sample protein concentration, mg/mL, should be determined by oneself;
W: sample mass, g;
N: Number of cells, 10^4;
T: Response time, 2h;
F: Dilution ratio.

Experimental examples:

1. Take 0.1006g of bamboo leaf was added with 1mL Extraction reagent, homogenized in ice bath, centrifuged, centrifuged and the supernatant was taken according to the measurement procedure. After determination with 96-well plate, calculate $\Delta A_T=A_T - A_C$ =0.380-0.293=0.087. By substituting ΔA into the standard equation y=1.5528x-0.0176, R^2=0.9998, calculate x=0.0674, Enzyme activity calculated by sample mass:
DAO activity（U/g weight）= x ×V_S÷(W÷V_{EV}×V_S) ÷T×F=0.5x÷ W×F=0.335 U/g weight
2. Take 0.1015g of Rat liver was added with 1mL Extraction reagent, homogenized in ice bath, centrifuged, centrifuged and the supernatant was taken according to the measurement procedure. After determination with 96-well plate, calculate $\Delta A_T=A_T - A_C$ =0.182-0.146=0.036. By substituting ΔA into the standard equation y=1.5528x-0.0176, R^2=0.9998, calculate x=0.0345, Enzyme activity calculated
by sample mass:
DAO activity（U/g weight）= x ×V_S÷(W÷V_{EV}×V_S) ÷T×F=0.5x÷ W×F=0.170 U/g weight

Related Products：

BC0020/BC0025	Malondialdehyde，MDA Content Assay Kit
BC0690/BC0695	Glucose oxidase (GOD) Assay Kit
BC1090/BC1095	Xanthine oxidase Assay Kit
BC1270/BC1275	Protein Carbonyl Assay Kit