TECHNICAL MANUAL

Diamine oxidase (DAO) Activity Assay Kit

- **SKU CODE:** MAES0214
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- ![img-0.jpeg](img-0.jpeg)

2. Intended use

This kit can be used to measure diamine oxidase (DAO) activity in serum, plasma, urine, animal tissue and cell samples.

3. Detection principle

Diamine oxidase (DAO, E.C.1.4.3.6) is widely distributed in animal tissues (intestinal mucosa, lung, liver, kidney, etc.), serum, plasma and cells. It is a highly active intracellular enzyme in the upper villi of human and mammalian small intestinal mucosa. It can regulate intracellular ion balance, affect conduction pathways and promote cell repair.

Detection principle: Diamine oxidase catalyzes amine substances to produce hydrogen peroxide, and hydrogen peroxide can react with a chromogenic substance to produce a colored product, which has a characteristic absorption peak at 460 nm. The activity of diamine oxidase can be calculated by measuring the rate of change in absorbance per unit time.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	2-8 °C, 12 months
Reagent 2	Substrate	1.5 mL × 2 vials	2-8 °C, 12 months, protected from light
Reagent 3	Enzyme Reagent	Powder × 1 vial	2-8 °C, 12 months, protected from light
Reagent 4	Chromogenic Agent	Powder × 1 vial	2-8 °C, 12 months, protected from light
Reagent 5	Stop Agent	10 mL × 1 vial	2-8 °C, 12 months
Reagent 6	100 mmol/L Standard	1 mL × 1 vial	2-8 °C, 12 months, protected from light

Item	Component	Size (96 T)	Storage
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments: Centrifuge, Incubator, Microplate reader (450-470 nm, optimum wavelength: 460 nm).

6. Reagent preparation

1. Preserve the enzyme reagent on ice, and equilibrate other reagents to room temperature before use.
2. **Preparation of enzyme stock solution:** Dissolve one vial of enzyme reagent with 1.5 mL double-distilled water and mix well. Store at 2-8°C for 7 days protected from light.
3. **Preparation of enzyme working solution:** For each well, prepare 100 µL of enzyme working solution (mix well 10 µL of enzyme stock solution and 90 µL of buffer solution). The enzyme working solution should be prepared immediately before use and kept on ice protected from light during detection.
4. **Preparation of chromogenic working solution:** Dissolve one vial of chromogenic agent with 1.9 mL double-distilled water. Store at 2-8°C for 7 days protected from light.
5. **Preparation of 5 mmol/L standard:** Dilute 15 µL of 100 mmol/L standard with 285 µL of double-distilled water. Prepare the fresh amount needed before use, and store at 2-8°C for 7 days protected from light.
6. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 5 mmol/L standard with double-distilled water to serial concentrations. The recommended dilution gradient is as follows: 0.0, 0.2, 0.5, 0.7, 1.0, 1.2, 1.5, 2.0 mmol/L.

7. Sample preparation

Serum and plasma: Detect directly. If the sample is turbid, centrifuge at 2000 g for 10 min, then take the supernatant for detection. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Homogenize 20 mg tissue in 180 µL buffer solution with a dounce homogenizer at 4°C.

3. Centrifuge at 10000 × g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

4. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cells:

1. Harvest the number of cells needed for each assay (initial recommendation 2×10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 2×10^6 cells in 200 µL buffer solution with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10000 × g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only): Human serum (2-4), Human plasma (1), Rat serum (1), Rat plasma (1), Mouse serum (1), Mouse plasma (1), 10% Rat liver tissue homogenate (1), 10% Rat kidney tissue homogenate (1), 10% Rat heart tissue homogenate (1), Human urine (1), HeLa Cells (1×10^6) (1).

Note: The diluent is buffer solution. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. Prevent the formation of bubbles when stop agent is transferred into the microplate.
2. If the detection system is turbid after adding stop agent, mix the reaction system.
3. If there is solid substance in stop agent, heat it at 60°C for 5 min until clear.

9. Operating steps

1. Standard well: Take 20 µL of standard solution with different concentrations to the corresponding wells. Control well: Take 20 µL of sample to the corresponding wells. Sample well: Take 20 µL of sample to the corresponding wells.

2. Add 20 µL of substrate and 100 µL of enzyme working solution to each well.

3. Add 30 µL of double-distilled water into the control wells. Add 30 µL of chromogenic working solution into the standard wells and sample wells.

4. Mix fully with microplate reader and incubate at 37°C for 30 min.

5. Add 50 µL of stop agent into each well (Please pipette slowly to avoid large bubbles).

6. Mix fully with microplate reader and incubate at 37°C for 5 min.

7. Measure the OD value of each well with microplate reader at 460 nm (Break the bubbles before measurement if there are any bubbles).

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve by using absolute OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

Definition: The amount of enzyme in 1 L of serum (plasma) that hydrolyzes the substrate to produce 1 µmol substance at 37°C for 1 min is defined as 1 unit.

DAO activity (U/L) = (ΔA_460 - b) / a ÷ T × f × 1000*

2. Tissue sample and cells sample:

Definition: The amount of enzyme in 1 g of tissue protein that hydrolyzes the substrate to produce 1 µmol substance at 37°C for 1 min is defined as 1 unit.

DAO activity (U/gprot) = (ΔA_460 - b) / a ÷ C_{pr} ÷ T × f × 1000*

[Note]

ΔA_460: OD_{Sample} - $OD_{Control}$.

T: The time of incubation reaction, 30 min.

C_{pr}: Concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

1000*: 1 mmol/L = 1000 µmol/L.

11. Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	18.4	62.5	112.0
%CV	5.6	5.2	4.2

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	18.4	62.5	112.0
%CV	6.4	7.2	7.4

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with each concentration performed 6 times in parallel to obtain an average recovery rate of 105%.

Recovery

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.35	0.94	1.3

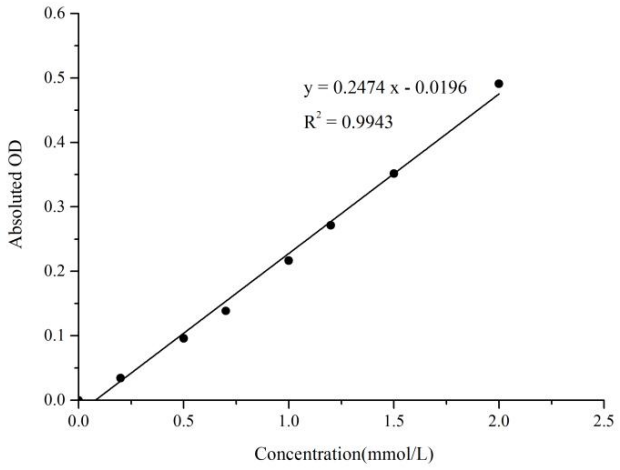
	Standard 1	Standard 2	Standard 3
Observed Conc. (mmol/L)	0.4	1.0	1.4
Recovery rate (%)	101	106	108

Sensitivity

The analytical sensitivity of the assay is 7.39 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve

Concentration (mmol/L)	OD value		Average OD	Absolute OD
0	0.065	0.064	0.065	0.000
0.2	0.098	0.100	0.099	0.035
0.5	0.161	0.160	0.161	0.096

Concentration (mmol/L)	OD value		Average OD	Absolute OD
0.7	0.198	0.208	0.203	0.139
1.0	0.280	0.282	0.281	0.217
1.2	0.336	0.336	0.336	0.272
1.5	0.413	0.419	0.416	0.352
2.0	0.556	0.555	0.556	0.491

Example Analysis

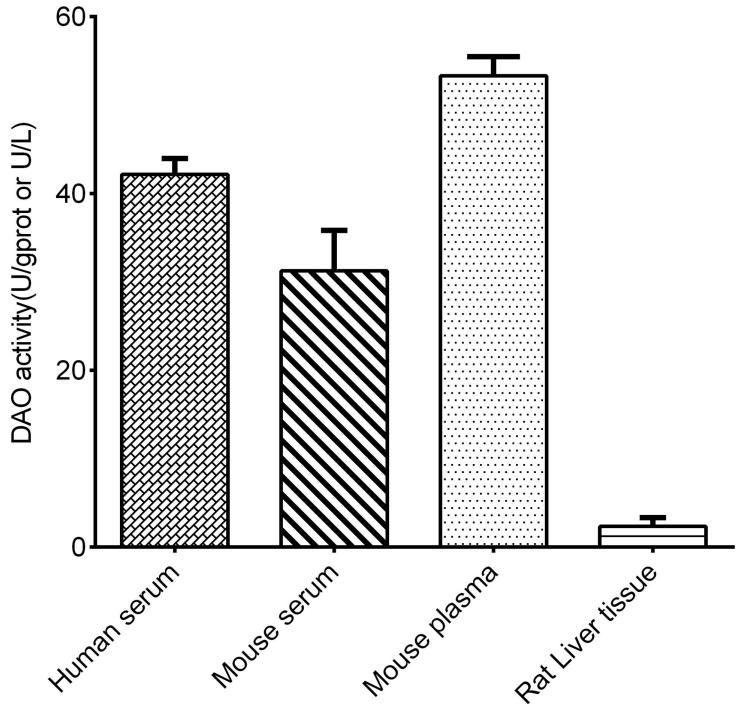
Example analysis:

For human serum, take 20 μL of human serum diluted 2-fold, and carry out the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.2474x - 0.0196, the average OD value of the control is 0.058, the average OD value of the sample is 0.194, and the calculation result is:

DAO activity (U/L) = (0.194 - 0.058 + 0.0196) ÷ 0.2474 ÷ 30 × 2 × 1000 = 41.93 U/L

Detect human serum (diluted 2-fold), mouse serum, mouse plasma and 10% rat liver tissue homogenate (the concentration of protein is 10.73 gprot/L) according to the protocol. The result is as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.