Technical Manual

Formate Colorimetric Assay Kit

- **Catalogue Code: MAES0095**
- **Size: 96T**
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

8.20-800 µmol/L

Sensitivity:

8.20 µmol/L

Storage:

-20°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Formic acid is the simplest carboxylic acid, whose chemical formula is CH2O2. It is often used as an antimicrobial/preservative in livestock feed. Formic acid can block some of the decay processes in feed, making the nutritional value of feed maintain longer. Under normal circumstances, the physiological concentration of formic acid is low and easy to metabolize, but in the case of methanol poisoning, the concentration can reach 5 mmoL/L. Similarly, long-term exposure to excessive levels of formaldehyde can also increase the content of formic acid in blood and urine.

3. Intended Use

This kit can be used to measure formate content in serum, plasma and animal tissue samples.

4. Detection Principle

Formic acid dehydrogenase (FDH) catalyzes the reaction of formic acid with NAD^+ to produce NADH. NADH, under the action of PMS, transfers electrons to WST-8 to produce the yellow product, which has a characteristic absorption peak at 450 nm. The NAD(P)H in the sample itself will cause certain background interference, so set the control well in the measurement process to eliminate such interference.

5. Kit components & storage

Item	Specification	Storage
Extracting Solution	60 mL × 2 vials	-20°C, 6 months
Substrate A	Lyophilized × 1 vial	-20°C, 6 months, avoid direct sunlight
Substrate B	Lyophilized × 1 vial	-20°C, 6 months, avoid direct sunlight
Chromogenic Agent	1.5 mL × 2 vials	-20°C, 6 months, avoid direct sunlight
Acid Agent	6 mL × 1 vial	-20°C, 6 months
Alkali Reagent	6 mL × 1 vial	-20°C, 6 months
Standard (10 mmol/L)	1.0 mL × 1 vial	-20°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (450 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

Prevent bubbles when adding samples. Break the bubbles before measurement if there are some bubbles.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **substrate A working solution:** Dissolve substrate A with 0.17 mL of extracting solution fully. Prepare the fresh solution before use and preserve it on ice for detection. The prepared solution can be stored at -20°C with avoid direct sunlight for 7 days.

3. Preparation of **substrate B working solution:** Dissolve substrate B with 0.3 mL of extracting solution fully. Prepare the fresh solution before use and preserve it on ice for detection. The prepared solution can be stored at -20°C with avoid direct sunlight for 7 days.

4. Preparation of **sample working solution:** Mix the extracting solution, substrate A working solution, substrate B working solution and chromogenic agent at a ratio of 8:1:1:10 fully. Prepare the fresh solution before use and store it with avoid direct sunlight.

5. Preparation of **control working solution:** Mix the extracting solution, substrate B working solution and chromogenic agent at a ratio of 9:1:10 fully. Prepare the fresh solution before use and store it with avoid direct sunlight.

8. Sample Preparation

1. Serum sample:

Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Tissue sample:
Accurately weigh the tissue sample, add extracting solution according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (8.20-800 μmol/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor
Human serum	1
Dog serum	1
Mouse serum	1
Horse serum	1
Porcine serum	1
Rat plasma	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat brain tissue homogenate	1

Note: The diluent is extracting solution;

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 450 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, sample wells; S1'- S40', control wells.

10. Operation Steps

Pretreatment of sample

1. Take 0.1 mL of liquid sample or tissue homogenate supernatant into 1.5 mL EP tube and add 0.15 mL of extracting solution, 0.05 mL of acid agent. Mix fully with vortex mixer for 30 s, centrifuge at 10000 g for 10 min at 4°C, then take the supernatant for measurement.

2. Take 0.15 mL of supernatant to 0.5 mL EP tube and add 0.05 mL of alkali reagent. Oscillate with vortex mixer for detection.

The preparation of standard curve

Dilute standard solution (10 mmol/L) with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 100, 200, 300, 400, 500, 600, 800 μmol/L.

The measurement of samples

1. **Sample well:** add 50 μL of sample into the corresponding wells.
   Standard well: add 50 μL of standard solution with different concentrations into the corresponding wells.
   Control well: add 50 μL of sample into the corresponding wells.

2. Add 50 μL of sample working solution into the sample wells and standard wells.

3. Add 50 μL of control working solution into the control wells.

4. Mix fully for 5 s with microplate reader and incubate at 37°C for 30-60 min. Measure the OD values of each well at 450 nm with microplate reader. The OD values of sample well recorded as A_2, the OD values of control well recorded as A_1, then $\triangle A = A_2 - A_1$.

Note: With the extension of incubation time, the color will grow deepen. Under the condition of the normal color of the sample, try to make the OD value at the highest point of the standard curve within the range of 1.5-2.0.

Operation Table

	Standard well	Sample well	Control well
Sample (μL)		50	50
Standards solution with different concentrations (μL)	50		
Sample working solution (μL)	50	50	
Control working solution (μL)			50
Mix fully for 5 s with microplate reader and incubate at 37°C for 30-60 min. Measure the OD values of each well at 450 nm with microplate reader. The OD values of sample well recorded as A_2, the OD values of control well recorded as A_1, then $\triangle A = A_2 - A_1$.			

11. Calculations

Plot the standard curve by using fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the fluorescence value of sample.

The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

Formate content (μmol/L) = (ΔA - b) ÷ a × 4*× f

2. Tissue sample:

Formate content (μmol/g wet weight)

= (ΔA - b) ÷ a× 4*× f× V÷W

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0)

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve;

ΔA: $\triangle A = A_2 - A_1$

4*: Dilution factor of liquid sample and tissue homogenate in the pretreatment of sample

f: Dilution factor of sample before test

V: The volume of extraction solution during tissue homogenate, 0.9 mL = 0.0009 L

W: Weight of sample, 0.1 g

12. Performance Characteristics

Detection Range	8.20-800 μmol/L
Sensitivity	8.20 μmol/L
Average recovery rate (%)	100
Average inter-assay CV (%)	3.9
Average intra-assay CV (%)	3.3

Analysis

Take 100 μL of human serum, carry the assay according to the operation table.

The results are as follows:

Standard curve: y = 0.0019 x – 0.0017, the average OD value of the sample (A_2) is 0.553, the average OD value of the control (A_1) is 0.470, then $\triangle A = A_2 - A_1$=0.083, and the calculation result is:

$$\textbf{Formate content (μmol/L)} = (0.083 + 0.0017) \div 0.0019 \times 4$$

$$= 178.32 \text{ μmol/L}$$

Email: info@assaygenie.com **Web:** www.assaygenie.com

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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