PRODUCT INFORMATION & MANUAL

Formate Assay Kit (Colorimetric)

NBP3-25798

For research use only.
Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

Formate Colorimetric Assay Kit

Catalog No: NBP3-25798

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 8.20 μmol/L

Detection range: 8.20-800 μmol/L

Average intra-assay CV (%): 3.3

Average inter-assay CV (%): 3.9

Verage recovery rate (%): 100

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲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.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extracting Solution	60 mL × 2 vials	-20℃ , 12 months
Reagent 2	Substrate A	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 3	Substrate B	Powder × 1 vial	-20℃ , 12 months, shading light
Reagent 4	Chromogenic Agent	1.5 mL × 2 vials	-20℃ , 12 months, shading light
Reagent 5	10 mmol/L Standard	1.0 mL × 1 vial	-20℃ , 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Microplate reader (450 nm), Pipettor, Water bath, Centrifuge

Reagents

Double distilled water

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

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

▲ The key point of the assay

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

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.

2. Preparation of reagent 2 working solution

Dissolve reagent 2 with 0.17 mL of reagent 1 fully. Prepare the fresh solution before use and preserve it on ice for detection. The prepared solution can be stored at -20℃ with shading light for 7 days.

3. Preparation of reagent 3 working solution

Dissolve reagent 3 with 0.3 mL of reagent 1 fully. Prepare the fresh solution before use and preserve it on ice for detection. The prepared solution can be stored at -20℃ with shading light for 7 days.

4. Preparation of sample working solution

Mix the reagent 1, reagent 2 working solution, reagent 3 working solution and reagent 4 at a ratio of 8:1:1:10 fully. Prepare the fresh solution before use and store it with shading light.

▲Sample preparation

1. Serum (Plasma):

Centrifuge with 10 KD ultrafiltration tube at 12000×g for 10 min. Take the filtrate for detect after ultrafiltration.

2. Tissue sample:

Accurately weigh the tissue sample, add reagent 1 according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge with 10 KD ultrafiltration tube at 12000×g for 10 min. Take the filtrate for detect after ultrafiltration.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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 reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operating steps

The preparation of standard curve

Dilute 10 mmol/L standard solution 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. Reference is as follows:

Number	Standard concentrations (μmol/L)	10 mmol/L Standard(μL)	Double distilled water (μL)
A	0	0	1000
B	100	10	990
C	200	20	980
D	300	30	970
E	400	40	960
F	500	50	950
G	600	60	940
H	800	80	920

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.
2. Add 50 μL of sample working solution into the sample wells and standard wells.
3. Mix fully for 5 s with microplate reader and incubate at 37℃ for 30 min. Measure the OD values of each well at 450 nm with microplate reader.

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 range of 1.5-2.0.

▲Summary operation table

	Standard well	Sample well
Sample (μL)		50
Standards solution with different concentrations (μL)	50	
Sample working solution (μL)	50	50
Mix fully and incubate at 37℃ for 30 min. Measure the OD values at 450 nm.		

▲Calculation

Plot the standard curve by using OD 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 OD value of sample.
The standard curve is: y= ax + b.

1. Serum (plasma) sample

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

2. Tissue sample

Formate content (μmol/g wet weight)= (ΔA - b) ÷ a × f × V ÷ W

Note:

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: OD_{Sample} – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0);;

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

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Appendix I Data

▲Example analysis

Take 50 μL of human serum and 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 is 0.422, the average OD value of the blank is 0.266, then ΔA = 0.422-0.266 = 0.156, and the calculation result is:

Formate content (μmol/L)=（ 0.156 +0.0017 ）÷0.0019 = 83 μmol/L