（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K1100-M

Specification：96T(80 samples)

Measuring instrument：Microplate reader（450 nm）

Detection range：8.20-800 μmol/L

Elabscience®Formate Colorimetric Assay Kit

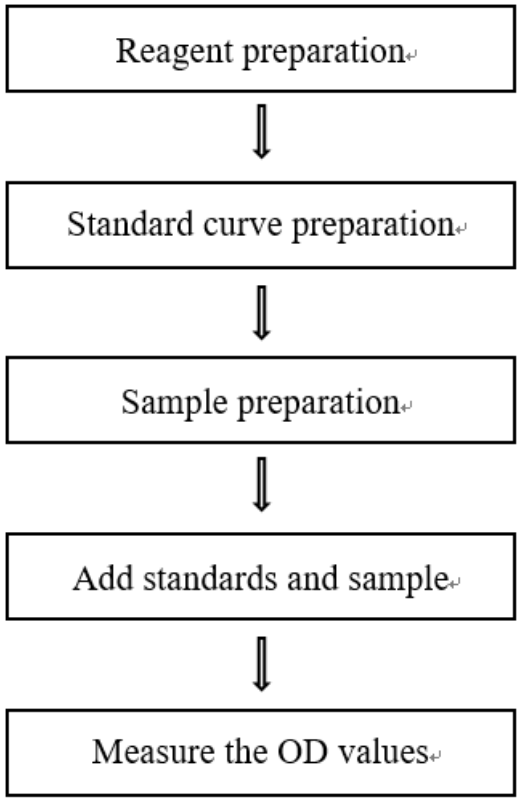
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

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

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	Size (96 T)	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	Plate Sealer	

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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of substrate A working solution:
Dissolve one vial of substrate A with 170 μL of extracting solution, mix well to dissolve. Keep substrate A working solution on ice during use. The substrate A working solution should be prepared on spot. Store at -20℃ for 7 days protected from light.

③ Preparation of substrate B working solution:
Dissolve one vial of substrate B with 300 μL of extracting solution, mix well to dissolve. Keep substrate B working solution on ice during use. The substrate B working solution should be prepared on spot. Store at -20℃ for 7 days protected from light.

④ Preparation of measuring working solution:
Before testing, please prepare sufficient measuring working solution according to the test wells. For example, prepare 100 μL of measuring working solution (mix well 40 μL of extracting solution, 5 μL of substrate A working solution, 5 μL of substrate B working solution and 50 μL of chromogenic agent). The measuring working solution should be prepared on spot. Keep measuring working solution on ice protected from light during use.

⑤ The preparation of standard curve:
Always prepare a fresh set of standards. Discard working standard dilutions after use.

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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**100**	**200**	**300**	**400**	**500**	**600**	**800**
10 mmol/L standard (μL)	0	20	40	80	100	120	140	160
Double distilled water (μL)	1000	990	980	970	960	950	940	920

Sample preparation

① Sample preparation

Serum and plasma: Centrifuge with 10 KD ultrafiltration tube at 12000×g for 10 min at 4℃. Take the filtrate for detect after ultrafiltration.

Tissue sample:

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

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extracting solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and centrifuge with 10 KD ultrafiltration tube at 12000×g for 10 min. Take the filtrate for detect after ultrafiltration.

② Dilution of sample

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. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

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

Operating steps

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 measuring 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 the range of 1.5-2.0.

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 absoluted OD value.

3. Plot the standard curve by using absoluted 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:

$$\text{Formate content (μmol/L)} = (\Delta A - b) \div a \times f$$

2. Tissue sample:

$$\text{Formate content (μmol/g wet weight)} = (\Delta A - b) \div a \times f \times V \div W$$

[Note]

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	16.50	135.00	264.00
%CV	3.6	3.2	3.1

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	16.50	135.00	264.00
%CV	4.1	3.8	3.8

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	125	362	554
Observed Conc. (μmol/L)	126.3	358.4	554.0
Recovery rate (%)	101	99	100

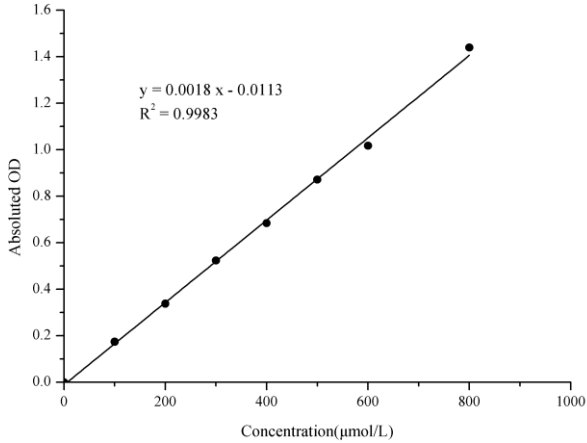
Sensitivity

The analytical sensitivity of the assay is 8.2 μmol/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.

2. 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), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**100**	**200**	**300**	**400**	**500**	**600**	**800**
Average OD	0.154	0.327	0.492	0.677	0.838	1.025	1.171	1.593
Absoluted OD	0.000	0.174	0.338	0.524	0.684	0.872	1.017	1.440



Appendix II Example Analysis

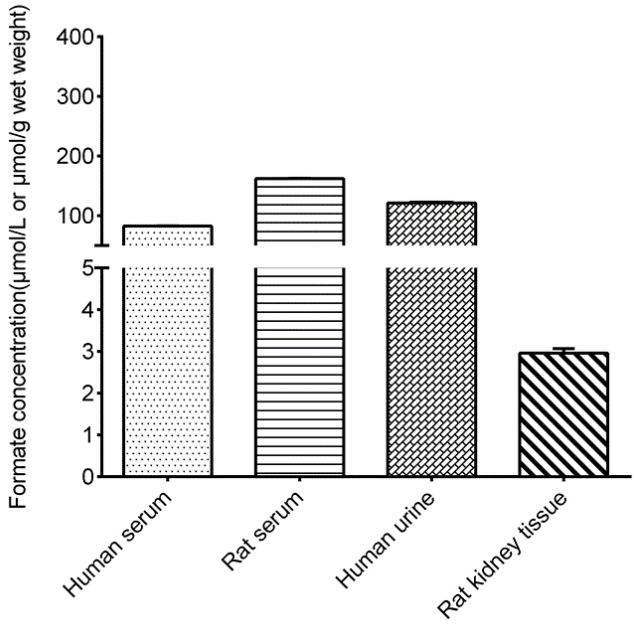
Example analysis：

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 △A = 0.422 – 0.266=0.156 , and the calculation result is:

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

Detect human serum, rat serum, human urine and 10% rat kidney tissue homogenate according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.