TECHNICAL MANUAL

Formate Colorimetric Assay Kit

- **SKU CODE:** MAES0095
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure the OD values

2. Intended use

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

3. 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 a 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 control wells are set in the measurement process to eliminate such interference.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extracting Solution	60 mL × 2 vials	-20°C, 12 months
Reagent 2	Substrate A	Powder × 2 vials	-20°C, 12 months, protect from light
Reagent 3	Substrate B	Powder × 1 vial	-20°C, 12 months, protect from light
Reagent 4	Chromogenic Agent	1.5 mL × 2 vials	-20°C, 12 months, protect from light
Reagent 5	10 mmol/L Standard	1.0 mL × 1 vial	-20°C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

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

Reagents:

Double distilled water

6. 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 fresh before each assay. Store at -20°C for up to 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 fresh before each assay. Store at -20°C for up to 7 days protected from light.
4. **Preparation of measuring working solution::** Before testing, prepare sufficient measuring working solution according to the number of test wells. For example, to prepare 100 µL of measuring working solution, thoroughly mix 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 fresh before each assay. Keep measuring working solution on ice and protected from light during use.
5. **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 create a serial concentration. The recommended dilution gradient is as follows: 0, 100, 200, 300, 400, 500, 600, 800 µmol/L.

7. Standard dilution table

Item	1	2	3	4	5	6	7	8
Concentration (µmol/L)	0	100	200	300	400	500	600	800

Item	1	2	3	4	5	6	7	8
10 mmol/L standard (μL)	0	10	20	30	40	50	60	80
Double distilled water (μL)	1000	990	980	970	960	950	940	920

8. Sample preparation

Sample preparation:

Serum and plasma: Centrifuge with 10 kDa ultrafiltration tube at 12,000×g for 10 min at 4°C. Take the filtrate for detection 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°C.

4. Centrifuge at 10,000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and centrifuge with 10 kDa ultrafiltration tube at 12,000×g for 10 min. Take the filtrate for detection after ultrafiltration.

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 perform a pretest to confirm the dilution factor.

9. Key points of the assay

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

10. 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 thoroughly for 5 seconds with microplate reader and incubate at 37°C for 30 min. Measure the OD values of each well at 450 nm with microplate reader.

11. 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 corresponding 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:

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

2. Tissue sample:

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

12. 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 (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.

Inter-assay Precision

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

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with 6 parallel measurements for each concentration to achieve an average recovery rate of 100%.

Recovery

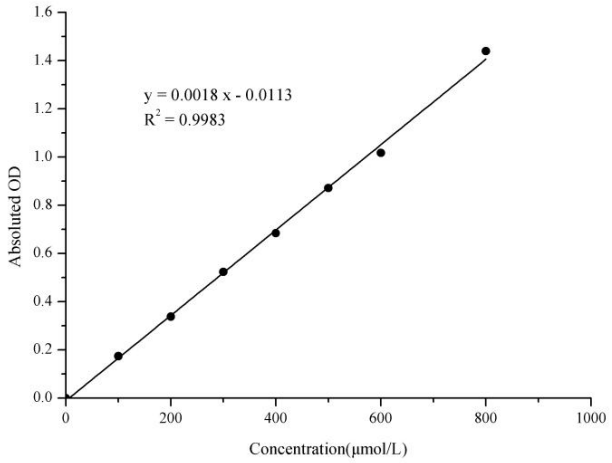
	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	125	362	554
Observed Conc. (µmol/L)	126.3	358.4	554.0
Recovery rate (%)	101	99	100

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.

Standard curve data

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 data

Concentration (µmol/L)	Average OD	Absolute OD
0	0.154	0.000
100	0.327	0.174
200	0.492	0.338

Concentration (μmol/L)	Average OD	Absolute OD
300	0.677	0.524
400	0.838	0.684
500	1.025	0.872
600	1.171	1.017
800	1.593	1.440

13. Example Analysis

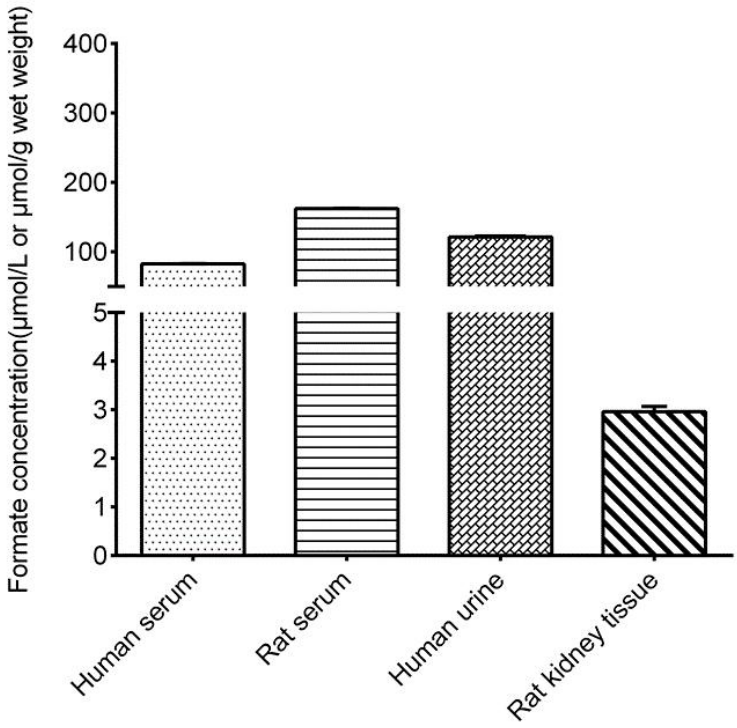
Example analysis:

Take 50 μL of human serum and perform the assay according to the operation procedure. The results are as follows:

Standard curve: y = 0.0019x – 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

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



14. 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. 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. The 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.