Formate

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1340

(Version 1.1A)

Detection and Quantification of Formate Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

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I. INTRODUCTION

Formate is a key intermediate in one-carbon metabolism, involved in purine and methylation synthesis under normal conditions. In methanol or formaldehyde exposure, formate accumulates and causes neurological and visual toxicity, serving as the primary pathogenic factor in methanol poisoning. Measuring formate levels aids in diagnosing methanol poisoning, assessing prognosis, and monitoring formaldehyde exposure.

Formate Colorimetric Microplate Assay Kit provides a convenient tool for sensitive and specific detection of Formate in a variety of samples. The kit is based on linked enzymatic reaction catalyzed oxidation of Formate, which generates NADH and reacts with dye reagent to form a colored product. The color intensity increase at 450nm is proportional to the amount of Formate in the sample.

II. KIT COMPONENTS

Component	100 Assays	Storage
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	6 ml x 1	4 °C
Enzyme Diluent	1 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C, keep in dark
Coenzyme	Powder x 1	-20 °C, keep in dark
Dye Reagent A	Powder x 1	4 °C, keep in dark
Dye Reagent B	1 ml x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer
9. Water bath

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 20 mmol/L of standard stock solution, store at 4 °C for 1-2 weeks or -20 °C for 3-4 months after reconstitution. Then dilute to 2 mmol/L standard top solution by adding 100 µl stock solution into 900 µl distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 2/1/0.5/0.25/0.125/0.0625/0.0312 mmol/L.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Enzyme Diluent to dissolve before use. Keep in dark and store at 4°C for 3-5 days or -20 °C for 2-3 months.

Coenzyme: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use. Keep in dark and store at 4°C for 1 week or -20 °C for 2-3 months.

Dye Reagent A: Add 10 ml distilled water to dissolve before use, mix. Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For serum, plasma or Cell culture media samples

Detect directly or dilute with Assay Buffer.

2. For urine samples

Dilute 2- to 10-fold using with Assay Buffer.

3. For tissue samples

Weigh out 0.05 g tissue, homogenize with 500 µl Assay Buffer on ice; centrifuged at 8000g 4 °C for 10 minutes, transfer the supernatant into a new centrifuge tube, keep it on ice for detection.

4. For cell samples

Collect cell into centrifuge tube, discard the supernatant after centrifugation, add 500 µl Assay Buffer for 500 × 10^4 cell, sonicate (with power 20%, sonication 2s, intervation 1s, repeat 30 times); Centrifuge at 8000g 4 °C for 10 minutes and transfer the supernatant into a new centrifuge tube; keep it on ice for detection.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Reaction Buffer	60 µl	60 µl	60 µl
Coenzyme	10 µl	10 µl	10 µl
Enzyme	10 µl	10 µl	10 µl
Dye Reagent A	100 µl	100 µl	100 µl
Dye Reagent B	10 µl	10 µl	10 µl
Mix and incubate at room temperature for 15 minutes, record absorbance measured at 450 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \ (mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.2 According to the quantity of cells

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay})} \ (\mu mol/10^4)$$

2.3 According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay})} \ (\mu mol/g)$$

2.4 According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}} \ (\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

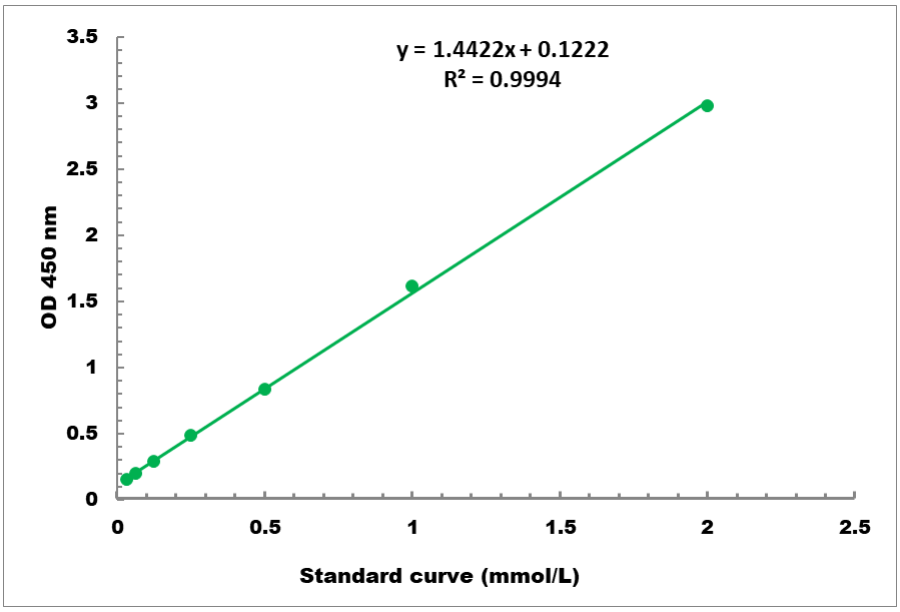
V_{Assay}: the volume of Assay Buffer, μl

W: the weight of sample, g

N: the quantity of cell, 10^4

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.02 mmol/L - 2 mmol/L