

Formate Assay Kit

(Catalog #K653-100; 100 assays; Store kit at -20°C)

I. Introduction:

Formic acid (HCOOH) is the simplest carboxylic acid and occurs naturally, notably in ant and bee venom. It is normally present at low levels in blood and urine (up to ~ 0.5 mM) but can be present in concentrations as high as 5 mM in acute methanol poisoning. Environmental exposure to formaldehyde can also elevate the blood and urine levels of formate. In the Formate Assay Kit, formate is oxidized to generate a product resulting in color formation ($\lambda = 450$ nm) proportional to formate concentration. The kit provides a convenient means for detecting formate in biological samples such as in serum or plasma, cells, culture and fermentation media. There is no need for pretreatment or purification of samples.

II. Kit Contents:

Components	K653-100	Cap Code	Part Number
Formate Assay Buffer	25 ml	WM	K653-100-1
Formate Enzyme Mix	1 vial	Green	K653-100-2
Formate Substrate Mix	1 vial	Red	K653-100-3
Formate Standard (100 mM)	100 μ l	Yellow	K653-100-4

III. Reagent Preparation and Storage Conditions:

Formate Enzyme Mix: Dissolve in 220 μ l Formate Assay Buffer. Pipette up and down to completely dissolve. Aliquot and store at -20°C. Use within two months.

Formate Substrate Mix: Reconstitute with 220 μ l of distilled H₂O and mix thoroughly. The solution is stable for 2 months at 4°C.

IV. Formate Assay Protocol:

1. **Standard Curve Preparations:** Dilute the Formate Standard to 1 mM by adding 10 μ l of the Formate Standard to 990 μ l of dH₂O, mix well. Add 0, 2, 4, 6, 8, 10 μ l into a series of wells on a 96 well plate. Adjust volume to 50 μ l/well with Formate Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Formate Standard.
2. **Sample Preparation:** Prepare test samples at 50 μ l/well with Formate Assay Buffer in a 96-well plate. If using serum sample, serum (0.5-10 μ l/assay, normal serum contains ~ 0-0.5 nmol/ μ l formate) can be directly diluted in the Formate Assay Buffer. Tissue (10 mg) or cells (1×10^6) can be homogenized in 100 μ l of Assay Buffer. Centrifuge in Eppendorf centrifuge at top speed for 10 min to remove insoluble materials. The soluble fraction may be assayed directly. We suggest using several doses of your sample to ensure the readings are within the standard curve range.
3. **Reaction Mix:** Mix sufficient reagent for the number of assays to be performed: For each well, prepare a total 50 μ l Reaction Mix containing the following components, mix well:

- 46 μ l Formate Assay Buffer
- 2 μ l Enzyme Mix
- 2 μ l Substrate Mix

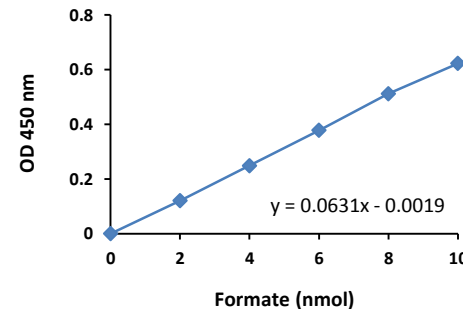
Note: NAD(P)H in samples may generate background readings. If significant amount of NAD(P)H is present in your sample, a sample background control can be performed by omitting Enzyme Mix in the Reaction Mix, then the sample background reading can be subtracted from the sample reading.

4. Add 50 μ l of the Reaction Mix to each well containing the Formate Standard or test samples, mix well.

5. Incubate the reaction for 60 min at 37°C.
6. Measure OD 450 nm in a microplate reader. The color is stable for at least 4 hrs.
7. **Calculations:** Correct background by subtracting the value derived from the 0 formate standard from all readings (Note: Background can be significant and must be subtracted from all standard and sample readings). Plot a Standard Curve of nmol/well vs. OD 450 nm. Apply the sample readings to the Standard Curve. Calculate the formate concentrations of the test samples:

$$C = La/Sv \text{ (nmol/}\mu\text{l or }\mu\text{M/ml or mM)}$$

Where: **La** is the formate amount (nmol) of your sample from standard curve.
Sv is the sample volume (μ l) added into the reaction well.
 Formic acid molecular weight: 46.02.



RELATED PRODUCTS:

- Cholesterol Assay Kit
- Glutathione Assay Kit
- Glucose Assay Kit
- Cell Proliferation Assay
- Cytotoxicity Assay
- CETP & PLTP Activity Assay Kits
- Pyruvate & Pyruvate Kinase Assay Kits
- Lactate Assay Kit
- Fatty Acid Assay Kit
- NAD/NADH & NADP/NADPH Assay Kits
- Triglyceride Assay Kit
- ATP & ADP Assay Kits
- Glycogen Assay Kit
- HDL/LDL Assay Kit
- beta-Hydroxybutyrate (β -HB) Assay Kit
- Ethanol Assay Kit
- Apoptosis Assay Related products

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range

Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.