Formate Colorimetric Assay Kit (#BN00877)

(Catalog #BN00877; 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 (λ = 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	BN00877	Cap Code	Part Number
Formate Assay Buffer	25 ml	WM	BN00877-1
Formate Enzyme Mix	1 vial	Green	BN00877-2
Formate Substrate Mix	1 vial	Red	BN00877-3
Formate Standard (100 mM)	100 µl	Yellow	BN00877-4

III. Reagent Preparation and Storage Conditions:

Formate Enzyme Mix: Dissolve in 220 µl 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 µl of distilled H_2O and mix thoroughly. The solution is stable for 2 months at 4°C.

IV. Formate Assay Protocol:

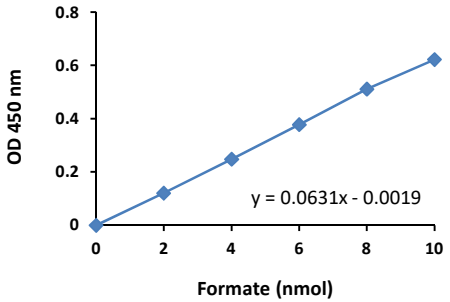
1. **Standard Curve Preparations:** Dilute the Formate Standard to 1 mM by adding 10 µl of the Formate Standard to 990 µl of dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells on a 96 well plate. Adjust volume to 50 µl/well with Formate Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Formate Standard.
2. **Sample Preparation:** Prepare test samples at 50 µl/well with Formate Assay Buffer in a 96-well plate. If using serum sample, serum (0.5-10 µl/assay, normal serum contains ~ 0-0.5 nmol/µl formate) can be directly diluted in the Formate Assay Buffer. Tissue (10 mg) or cells (1×10^6) can be homogenized in 100 µl 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 µl Reaction Mix containing the following components, mix well:

   46 µl Formate Assay Buffer
   2 µl Enzyme Mix
   2 µl 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 µl 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 (nmol/µl or µM/ml or mM)

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



FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		