Technical Bulletin

Formate Assay Kit

Catalog Number MAK491

Product Description

Formate ($HCOO^-$) is the anion derived from formic acid, the simplest carboxylic acid. It is also the metabolic byproduct of formaldehyde metabolism in the human body, and the eventual metabolic byproduct of methanol, which is first broken down to formaldehyde. At high levels, formate is neurotoxic to the central nervous system and can cause blindness, coma, and death. Although naturally present in the body at low levels, elevated levels of formate may be used as an indicator of formaldehyde exposure and methanol poisoning.

The Formate Assay Kit is based on the formate dehydrogenase-catalyzed oxidation of formate, which generates carbon dioxide and NADH that reduces a formazan (MTT) dye. The intensity of the reduced MTT, measured at 565 nm, is directly proportional to formate concentration in the sample.

The linear detection range of the kit is 0.050 – 5 mM formate. The kit is suitable for the determination of formate in biological samples such as serum and urine.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalog Number MAK491A
- MTT/NAD 1 mL
  Catalog Number MAK491B
- Enzyme A 120 µL
  Catalog Number MAK491C
- Enzyme B 120 µL
  Catalog Number MAK491D
- Standard (20 mM Formate) 0.5 mL
  Catalog Number MAK491E

Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Spectrophotometric multiwell plate reader.
- Clear flat bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store all components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to assay temperature (room temperature or 37 °C) prior to use. Keep thawed Enzyme A and Enzyme B on ice during the assay.

Procedure

All samples and standards should be run in duplicates.

Sample Preparation

Clear and slightly colored samples can be assayed directly.

Biological fluid samples (for example, urine or serum) can be assayed directly. Centrifuge samples prior to assay to remove any particulates. Appropriate dilution in purified water may be required.

For unknown Samples, perform a pilot experiment by testing several dilutions to ensure the readings are within the linear detection range of the kit.

Transfer 10 µL of each Sample into separate wells of a 96-well plate.

Standard Curve Preparation

1. Prepare a 1 mM Formate Standard by mixing 10 µL of 20 mM Formate Standard and 190 µL of purified water.
2. Prepare formate Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Formate Standards

Well	1 mM Standard	Purified water	Formate (mM)
1	100 µL	-	1.0
2	60 µL	40 µL	0.6
3	30 µL	70 µL	0.3
4	-	100 µL	0

3. Mix well and transfer 10 µL of each Standard into separate wells of a clear 96-well plate.

Working Reagent

1. Mix enough reagent for the number of assays to be performed. For each well, prepare 105 µL of Working Reagent according to Table 2.

Table 2.
Preparation of Working Reagent

Reagent	Volume
Assay Buffer	95 µL
MTT/NAD	8 µL
Enzyme A	1 µL
Enzyme B	1 µL

2. Add 90 µL of Working Reagent to each Standard and Sample well. Tap plate to mix.

Measurement

Incubate the plate for 60 minutes at room temperature. Read optical density (OD) at 565 nm.

Results

1. Calculate ΔOD by subtracting the OD reading of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD against standard concentrations and determine the slope of the standard curve.
3. Calculate the Formate concentration of the Sample:

$$\text{Formate (mM)} = \frac{\text{OD}_{\text{Sample}} - OD_{Blank}}{\text{Slope } (\text{mM}^{-1})} \times DF$$

where:

$\text{OD}_{\text{Sample}}$ = Optical density reading of Sample

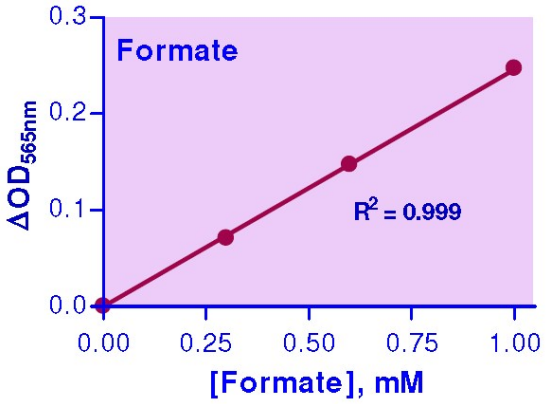
OD_{Blank} = Optical density reading of Blank (Standard #4)

DF = Sample dilution factor (DF = 1 for undiluted Samples)

If the Sample ΔOD values are higher than the ΔOD value for the 1.0 mM Standard, dilute Sample in purified water and repeat the assay. Multiply the results by the dilution factor.

Conversions: 1 mM formate equals 4.5 mg/dL or 45 ppm.

Typical Formate Standard Curve



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mak491pis Rev 09/22