ab111748

Formate Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Formate levels in various samples.

View kit datasheet: www.abcam.com/ab111748
(use www.abcam.cn/ab111748 for China, or www.abcam.co.jp/ab111748 for Japan)

This product is for research use only and is not intended for diagnostic use.

Version 4a, Last Updated 29 May 2025

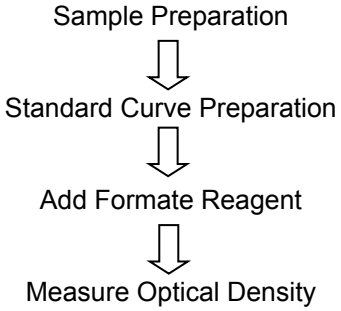
Table of Contents

1. Overview

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 Abcam's 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.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer 2	25 mL
Enzyme Mix IX	1 vial
Developer Solution III	1 vial
Formate Standard	0.1 mL

PLEASE NOTE: Assay Buffer 2 was previously labelled as Assay Buffer II and Formate Assay Buffer, and Enzyme Mix IX as Formate Enzyme Mix (Lyophilized), and Developer Solution III as Formate Substrate Mix (Lyophilized). The composition has not changed.

* Store kit at -20°C.

ENZYME MIX IX: Dissolve in 220 µl Assay Buffer 2. Pipette up and down to completely dissolve. Aliquot and store at -20°C. Use within two months.

DEVELOPER SOLUTION III: Reconstitute with 220 µl of distilled H_2O and mix thoroughly. The solution is stable for 2 months at +4°C.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

Prepare test samples at 50 µl/well with Assay Buffer 2 in a 96- well plate.

a. **For serum samples:** Serum (0.5-10 µl/assay, normal serum contains ~ 0-0.5 nmol/µl formate) can be directly diluted in the Assay Buffer 2.

b. **For tissue or cell samples:** Tissue (10 mg) or cells (1 x 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.

2. Standard Curve Preparation:

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 Assay Buffer 2 to generate 0, 2, 4, 6, 8, 10 nmol/well of the Formate Standard.

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:

Assay Buffer 2	46 µl
Enzyme Mix IX	2 µl
Developer Solution III	2 µl

Add 50 µl of the Reaction Mix to each well containing the Formate Standard or test samples, mix well. Incubate the reaction for 60 min at 37°C.

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 IX in the Reaction Mix, then the sample background reading can be subtracted from the sample reading.

4. Measurement: Measure OD_{450nm} in a microplate reader. The color is stable for at least 4 hours.

5. Data Analysis

Correct background by subtracting the value derived from the zero formate standard from all readings. Background can be significant and must be subtracted from all standard and sample readings.

Plot a Standard Curve of nmol/well vs. OD_{450nm}. Apply the sample readings to the Standard Curve.

Calculate the formate concentrations of the test samples:

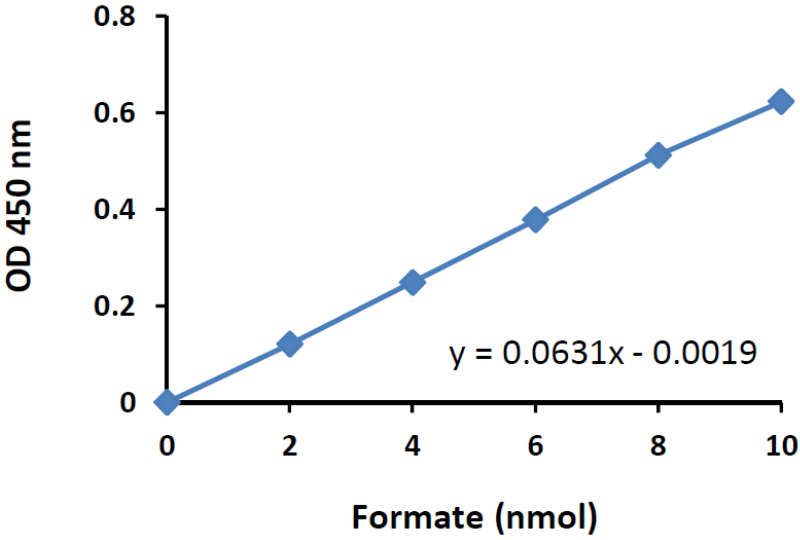
Concentration = La / Sv (nmol/µl, or µmol/ml, or mM)

Where:

La is the amount of Formate (nmol) of your sample from the standard curve.

Sv is the sample volume (µl) added to the reaction well.

Formate molecular weight: 46.02 g/mol.



Formate Standard Curve: Performed following the kit protocol.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)