Product Manual

Formate Assay Kit (Colorimetric)

Catalog Number

MET-5133 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Formic acid is the simplest carboxylic acid with chemical formula CH_2O_2. It is often used as an antibacterial agent/preservative in livestock feed. In some countries it is applied to fresh hay in order to promote lactic acid fermentation and to lower butyric acid formation. Formic acid can block certain decay processes in feed which results in the feed retaining its nutritive value longer, and is therefore often employed to preserve cattle winter feed. It is sometimes added to poultry feed to kill *E. coli* bacteria. Formic acid is also used to tan leather, dye textiles, remove limescale, and clean toilet bowls. Beekeepers use formic acid to kill mites that can destroy bee colonies. In humans at low physiological concentrations, formic acid is metabolized easily and removed by the body (normal urine levels are about 280 µM). At higher doses, however, it can be toxic; in methanol poisoning it is the formic acid and formaldehyde metabolites rather than methanol that cause optic nerve damage resulting in blindness. Long term formic acid exposure in humans may cause kidney damage as well as the development of a skin allergy that appears when re-exposure to the chemical.

Assay Principle

The Formate Assay Kit is a sensitive, quantitative colorimetric assay for formate in a variety of sample types. The provided reagents are sufficient for the evaluation of 100 assays*. The unknown samples or formate standards are added to a 96 well plate followed by the Colorimetric Probe Mix containing WST-1, an electron mediator, and Formate Dehydrogenase (FDH). During a brief incubation the WST-1 is converted to the formazan form (Figure 1) and the absorbance of the plate is read at 450 nm. The content of formate in the unknown samples is determined by comparison with a predetermined formate standard curve. The kit has a detection sensitivity limit of 12.5 µM formate.

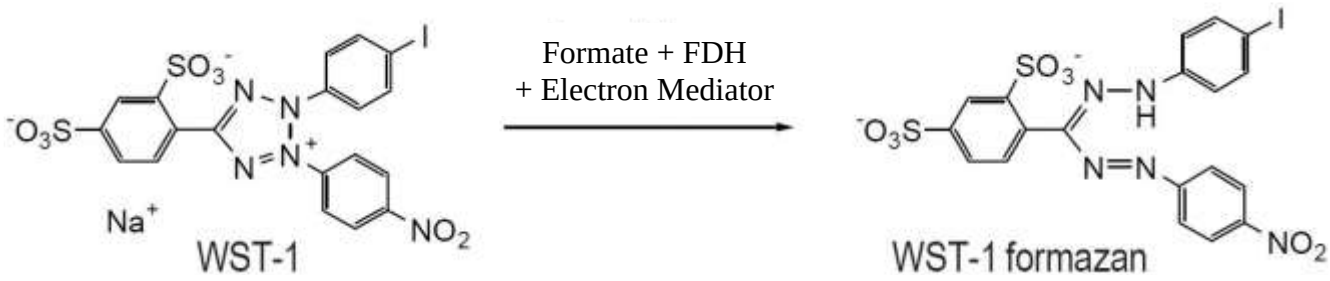

Figure 1. Assay Principle.

Note: Each sample replicate requires 2 assays, one treated with formate dehydrogenase (+FDH) and one without (-FDH). Formate levels are calculated from the difference in OD readings from the 2 wells.

Related Products

1. MET-5080: Glutamate Assay Kit (Colorimetric)
2. MET-5054: L-Amino Acid Assay Kit (Colorimetric)
3. MET-5056: Branched Chain Amino Acid Assay Kit
4. MET-5070: Glycine Assay Kit
5. MET-5073: Tyrosine Assay Kit
6. MET-5119: Malate Assay Kit (Colorimetric)
7. MET-5151: S-Adenosylhomocysteine (SAH) ELISA Kit
8. MET-5152: S-Adenosylmethionine (SAM) ELISA Kit
9. STA-341: OxiSelect™ Catalase Activity Assay Kit
10. STA-670: Homocysteine ELISA Kit

Kit Components

1. 10X Colorimetric Probe (Part No. 51331C): Two 1 mL amber vials.
2. Formate Standard (Part No. 51332C): One 500 µL vial at 80 mM.
3. 5X Assay Buffer (Part No. 51333A): One 30 mL bottle.
4. 50X NAD+ (Part No. 51334D): One 400 µL vial.
5. Formate Dehydrogenase (50X) (Part No. 51335D): One 400 µL vial at 5 U/mL

   Note: One unit (U) formate dehydrogenase will oxidize 1 µmol of formic acid to CO2 in 1 minute at +25 °C and pH 7.6.

Materials Not Supplied

1. Distilled or deionized water
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Standard 96-well clear microtiter plate
5. Multichannel micropipette reservoir
6. 37°C Incubator
7. Microplate reader capable of reading at 450 nm (620 nm as optional reference wavelength)

Storage

Upon receipt, store the 5X Assay Buffer at room temperature. Store the 50X NAD+ and Formate Dehydrogenase at -80°C. Store all remaining components at -20ºC. The 10X Colorimetric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

Note: All reagents must be brought to room temperature prior to use.

- 1X Assay Buffer: Dilute the stock 5X Assay Buffer 1:5 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store at room temperature.
- Reaction Mix: Dilute the 10X Colorimetric Probe, the Formate Dehydrogenase (50X) and the 50X NAD+ to 1X concentration in 1X Assay Buffer. For example, for 20 assays add 400 µL of 10X Colorimetric Probe, 80 µL of Formate Dehydrogenase (50X), and 80 µL of 50X NAD+ to 3.48 mL of 1X Assay Buffer.

  Note: Scale the described example up or down appropriately and prepare only enough for immediate use.
- Control Mix: Dilute both the 10X Colorimetric Probe and the 50X NAD+ to 1X concentration in 1X Assay Buffer. For example, for 20 assays add 400 µL of 10X Colorimetric Probe, and 80 µL of 50X NAD+ to 3.52 mL of 1X Assay Buffer.

  Note: Scale the described example up or down appropriately and prepare only enough for immediate use.

Preparation of Samples

Notes: All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with unknown samples.

- Cell culture supernatants: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary. Prepare the Formate standard curve in the same non-conditioned media.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates can be assayed undiluted or diluted as necessary in deionized water.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary in deionized water.

Preparation of Standard Curve

Prepare fresh Formate standards before use. Use the 80 mM Formate Standard to prepare a series of the remaining Formate standards according to Table 1 below.

Standard Tubes	80 mM Formate Standard (µL)	1X Assay Buffer (µL)	Formate (µM)
1	5	495	800
2	250 of Tube #1	250	400
3	250 of Tube #2	250	200
4	250 of Tube #3	250	100
5	250 of Tube #4	250	50
6	250 of Tube #5	250	25
7	250 of Tube #6	250	12.5
8	0	250	0

Table 1. Preparation of Formate Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each sample replicate requires two paired wells, one to be treated with FDH (Reaction Mix) and one without the enzyme (Control Mix) to measure endogenous sample background.
2. Add 50 µL of each sample (Formate standard or unknown) into wells of a 96 well plate.
3. Add 200 µL of Reaction Mix to the standards and to one half of the paired sample wells, and mix the well contents thoroughly.
4. Add 200 µL of Control Mix to the other half of the paired sample wells and mix thoroughly.
5. Incubate at 37°C for 60 minutes in an incubator.
6. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Calculation of Results.

1. Determine the average absorbance values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without FDH (-FDH) from the sample well values containing enzyme (+FDH) to obtain the difference. The absorbance difference is due to the enzyme FDH activity:

$$\Delta A = A_{(+FDH)} - A_{(-FDH)}$$

5. Compare the change in absorbance ΔA of each sample to the standard curve to determine and extrapolate the quantity of formate present in the sample. Only use values within the range of the standard curve.

Example of Results

The following figures demonstrate typical Formate Assay (Colorimetric) results. One should use the data below for reference only. This data should not be used to interpret actual results.

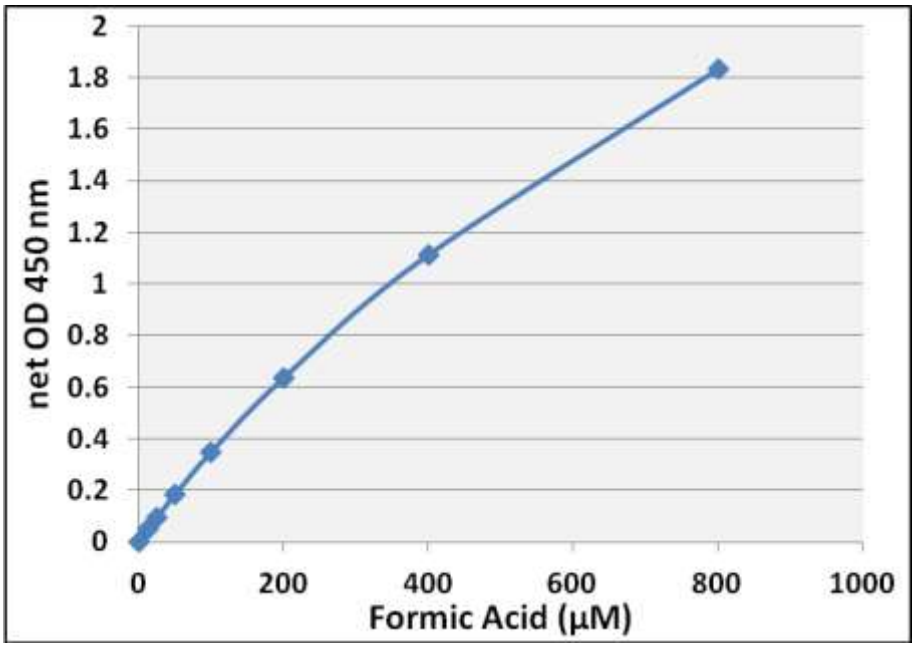

Figure 2. Formate Standard Curve.

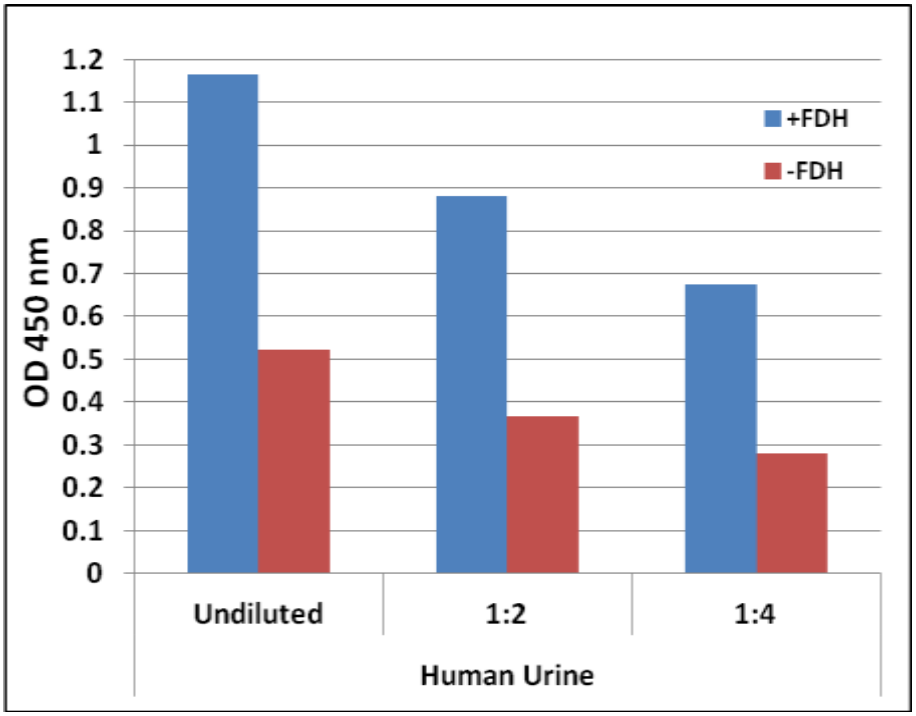

Figure 3. Detection of Formate in Urine. Human urine was assayed according to the kit protocol.

References

1. Griggs JP and Jacob JP (2005). *J. Appl. Poultry Res*. **14**: 750.
2. Garcia, V; Catala-Gregori, P; Hernandez, F; Megias, M. D; Madrid, J (2007). *J. Appl. Poultry Res*. **16**: 555.
3. Hoppe H.; Ritter W.; and Stephen EWC (1989). *Am. Bee J*. **129**:739–42.
4. Boeniger MF (1987). *Am. Ind. Hyg. Assoc. J.* **48**:900-908.
5. Sadun AA (2002). *J. Neurol. Neurosurg. Psych*. **72**: 423–425.

Warranty

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