# Formate Assay Kit (Colorimetric)

Formate Assay Kit (Colorimetric) is a detection kit for the quantification of Formate in serum, plasma, urine, cell lysate and cell culture supernatants.

Catalog number: ARG82658

Package: 100 assays

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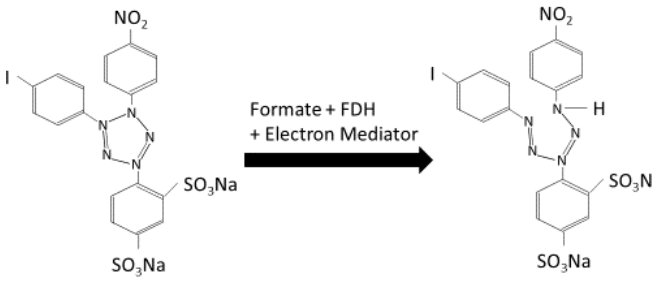
## INTRODUCTION

Formate (IUPAC name: methanoate) is the anion derived from formic acid. Its formula is represented in various equivalent ways: HCOO− or CHOO− or HCO2−. It is the product of deprotonation of formic acid. It is the simplest carboxylate anion. A formate (compound) is a salt or ester of formic acid. [Provide by Wikipedia: Formate]

## PRINCIPLE OF THE ASSAY

This Formate Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of formate present in serum, plasma, urine, cell lysates and cell culture supernatants. The assay is based on the enzyme driven reaction. The 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 and the absorbance of the plate is read at 450 nm. The concentration of formate in the samples is then determined by comparing the absorbance of samples to the standard curve. (See the Figure below)




**WST-1** **Formazan**

## MATERIALS PROVIDED & STORAGE INFORMATION

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 -20°C. The 10X Colorimetric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

| Component | Quantity | Storage information |
|---|---|---|
| 5X Assay Buffer | 30 mL | Room temperature |
| Formate Standards (80 mM) | 500 µL | -20°C |
| 10X Colorimetric Probe (amber tube) | 2 X 1 mL | -20°C |
| 50X NAD+ | 400 µL | -80°C |
| 50X Formate Dehydrogenase (5U/mL) | 400 µL | -80°C |

**Note:** One unit (U) formate dehydrogenase will oxidize 1 µmol of formic acid to $CO_2$ in 1 minute at 25°C and pH 7.6.

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm. (620 nm as optional reference wavelength)
- Standard 96-well microplate
- 37°C Incubator
- Deionized or Distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir

## TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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 -20°C. The 10X Colorimetric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.
- All reagents must be brought to room temperature prior to use.
- 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.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

**Cell Culture Supernatant:** To remove insoluble particles, centrifuge at 10,000 x g for 10 minutes at 4°C. The supernatant should be assayed directly or diluted as necessary in deionized water. Prepare the Formate Standard curve in the same non-conditioned medium.

**Cell Lysates:** Wash cells 3 times with cold 1X PBS prior to lysis. Re-suspend cells at 1-2 x $10^6$ cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge at 10,000 x g for 10 minutes at 4°C to remove the debris. Cell lysates can be assayed directly or diluted as necessary in deionized water..

**Plasma:** Collect blood with EDTA, heparin or citrate and centrifuge at 2,000 x g and 4°C for 10 minutes. The supernatant should be assayed directly or diluted as necessary in deionized water.

**Serum:** Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2,500 x g for 20 minutes. The supernatant should be assayed directly or diluted as necessary in deionized water.

**Urine:** To remove insoluble particles, centrifuge at 10,000 x g for 10 minutes at 4°C. The supernatant should be assayed directly or diluted as necessary in deionized water.

**Note:**

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum / plasma sample.

3. 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 samples.

## REAGENT PREPARATION

- 1X Assay Buffer: Dilute the 5X Assay Buffer into deionized water to yield 1X Assay Buffer. (E.g., add 15 mL of 5X Assay Buffer into 60 mL of deionized water to a final volume of 75 mL) Mix to homogeneity. Store the 1X Assay Buffer at room temperature.
- Reaction Mix: Dilute the 10X Colorimetric Probe, the 50X Formate Dehydrogenase and the 50X NAD+ to 1X concentration in 1X Assay Buffer. (E.g., for 20 assays, add 400 µL of 10X Colorimetric Probe, 80 µL of 50X Formate Dehydrogenase and 80 µL of 50X NAD+ to 3.44 mL of 1X Assay Buffer)
- Control Mix: Dilute the 10X Colorimetric Probe, the 50X Formate Dehydrogenase and the 50X NAD+ to 1X concentration in 1X Assay Buffer. (E.g., 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 Reaction Mix / Control Mix up or down appropriately and prepare only enough for immediate use.
- Standards: Prepare fresh Formate Standards before use. Use the 80 mM Formate Standard to prepare a series diluted Formate Standards according to the table below.

| Standard tube | Final Formate conc. (µM) | Volume of 1X Assay Buffer (µL) | Volume of 80 mM Formate Standard (µL) |
|---|---|---|---|
| S1 | 800 | 495 | 5 |
| S2 | 400 | 250 | 250 of S1 |
| S3 | 200 | 250 | 250 of S2 |
| S4 | 100 | 250 | 250 of S3 |
| S5 | 50 | 250 | 250 of S4 |
| S6 | 25 | 250 | 250 of S5 |
| S7 | 12.5 | 250 | 250 of S6 |
| S0 | 0 | 250 | 0 |

## ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

**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.

1. Add **50 µL** of **samples** or serial **diluted Formate Standards** into 96-well microplate.
2. Add **200 µL** of **Reaction Mix** to the Standards and to one half of the paired sample wells, and mix the well contents thoroughly.
3. Add **200 µL** of **Control Mix** to the other half of the paired sample wells and mix thoroughly.
4. Incubate for **60 minutes** at **37°C** in the dark.
5. Read the plate with a microplate reader at **O.D. 450 nm**.

## CALCULATION OF RESULTS

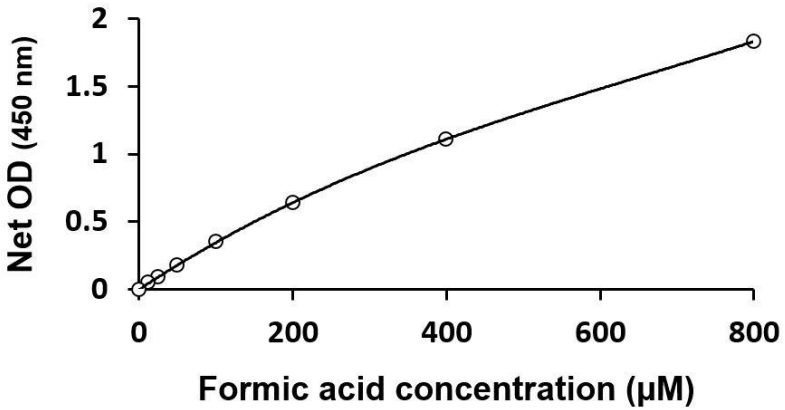
1. Calculate the average absorbance value for each set of Standards, Control and samples. Subtract the average Standard 0 value from itself and all standard and sample values. This is the corrected absorbance value.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Subtract the sample well values without FDH (Control Mix) from the sample well values containing enzyme (Reagent Mix) to obtain the difference. The absorbance difference is due to the FDH activity:

$$\Delta A = (A_{\text{Reagent mix}}) - (A_{\text{Control mix}})$$

4. 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 TYPICAL STANDARD CURVE

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




## QUALITY ASSURANCE

### Sensitivity

12.5 µM