

## EnzyChrom™ Formaldehyde Assay Kit (EHCHO-100)

### Quantitative Colorimetric Formaldehyde Determination

#### DESCRIPTION

**FORMALDEHYDE** (methanal) is the simplest aldehyde. It is widely employed in industry for a wide range of applications. Formaldehyde is also used as a disinfectant and is a commonly utilized tissue fixative and embalming agent. Formaldehyde is naturally present in all tissues and body fluids. Recently, it has been shown that some cancer types exhibit elevated formaldehyde levels. Increased formaldehyde concentration in urine has been associated with prostate and bladder cancer. Thus, measuring formaldehyde in urine can be a very useful tool when studying cancer.

BioAssay Systems' formaldehyde assay kit is based on formaldehyde dehydrogenase catalyzed oxidation of formaldehyde, which generates formate and NADH that reduces a formazan (MTT) dye. The intensity of the reduced MTT, measured at 565 nm, is directly proportional to formaldehyde concentration in the sample.

#### KEY FEATURES

**Fast and sensitive.** Use of 50 µL sample. Linear detection range 1.2 to 500 µM Formaldehyde in 96-well plate assay.

**Convenient.** The procedure involves adding a single working reagent and reading the absorbance after 30 minutes. Room temperature assay.

**High-throughput.** "Add-mix-read" type assay. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

#### APPLICATIONS

**Direct Assays:** formaldehyde in biological samples, food, beverages, and the environment

#### KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

<b>Assay Buffer:</b>	15 mL	<b>NAD/MTT:</b>	1.0 mL
<b>Enzyme A:</b>	120 µL	<b>Standard:</b>	100 µL 10 mM Formaldehyde
<b>Enzyme B:</b>	120 µL		

**Storage conditions.** The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

**Precautions:** reagents are for research use only. Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Safety Data Sheet for detailed information.

#### PROCEDURE

**Sample Preparation:** clear and slightly colored samples can be assayed directly. It is prudent to test several dilutions to determine an optimal dilution factor *n*.

*Biological fluid samples (e.g. urine & serum)* can be assayed directly, after centrifuging first to remove any particulates. Appropriate dilution in distilled water may be required.

#### Procedure using 96-well plate

- Standards.** Prepare 0.5 mL 500 µM Standard by mixing 25 µL of the Standard (10 mM) and 475 µL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + H <sub>2</sub> O	Formaldehyde (µM)
1	200 µL + 0 µL	500
2	120 µL + 80 µL	300
3	60 µL + 140 µL	150
4	0 µL + 200 µL	0

- Transfer 50 µL standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 50 µL of each sample into separate wells.
- Prepare sufficient Working Reagent (WR) for all sample and standard wells by mixing, for each well: 150 µL Assay Buffer, 10 µL MTT/NAD, 1 µL Enzyme A, and 1 µL Enzyme B.

Add 150 µL WR to the *four Standards* and the *Sample Wells*. Tap plate to mix briefly and thoroughly. Incubate 30 minutes at room temperature.

- Read optical density at 565 nm (520-600 nm).

#### CALCULATION

Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the formaldehyde concentration of Sample,

$$[\text{Formaldehyde}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Slope } (\mu\text{M}^{-1})} \times n \text{ (}\mu\text{M)}$$

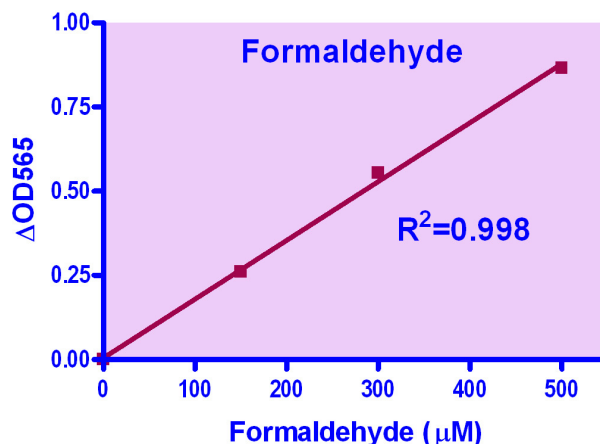
OD<sub>SAMPLE</sub> and OD<sub>BLANK</sub> are optical density readings of the Sample and Water Blank (#4), respectively. *n* is the sample dilution factor.

*Note: if the sample OD value is higher than OD for the 500 µM formaldehyde standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.*

**Conversions:** 1 µM formaldehyde equals 30 ppb.

#### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.



Standard Curve in 96-well plate assay in water

#### LITERATURE

- Ai, L., et al. (2019). Endogenous formaldehyde is a memory-related molecule in mice and humans. *Communications Biology*, 2(1), 446.
- Zhai, R et al (2016). Evidence for Conversion of Methanol to Formaldehyde in Nonhuman Primate Brain. *Analytical Cell Pathology (Amsterdam)*: 4598454.
- Yang, M et al (2014). Alzheimer's disease and methanol toxicity (part 2): lessons from four rhesus macaques (Macaca mulatta) chronically fed methanol. *J Alzheimers Dis.* 41(4): 1131-1147.

