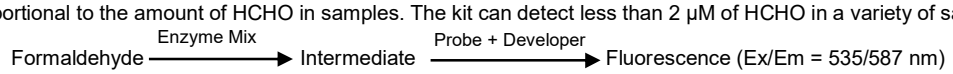
Formaldehyde Assay Kit (#BN01021)

(Catalog # BN01021; 100 assays; Store at -20°C)

I. Introduction:

Formaldehyde (HCHO) is the simplest aldehyde and one of the most often used organic compounds in industrial processes due to its high reactivity with a variety of chemicals. Endogenous HCHO can be produced in organisms via metabolic processes while exogenous HCHO can be absorbed after oral, dermal or inhalation exposure. HCHO is highly toxic due to its capacity to covalently bind to macromolecules, such as DNA, and is well known for its neurotoxicological effects. Exposure to relatively high levels of HCHO is believed to cause leukemia, nose and nasopharyngeal cancer, etc.; however the epidemiological evidence is unclear. It can also affect memory and learning capacity. Assay Genie's Formaldehyde Assay kit provides a simple, sensitive, and high-throughput adaptable assay that detects biologically relevant concentrations of HCHO in various fluids and tissues. The assay is based on the oxidation of HCHO, producing a stable fluorescent signal, which is directly proportional to the amount of HCHO in samples. The kit can detect less than 2 µM of HCHO in a variety of samples.



II. Application:

- Measurement of Formaldehyde in various biological samples

III. Sample Type:

- Biological fluids such as serum, plasma, saliva & urine
- Animal tissues & cells

IV. Kit Contents:

Components	BN01021	Cap Code	Part Number
HCHO Assay Buffer	25 ml	WM	BN01021-1
Formaldehyde Assay Kit	0.4 ml	Blue	BN01021-2
HCHO Enzyme Mix (Lyophilized)	1 vial	Green	BN01021-3
HCHO Developer (Lyophilized)	1 vial	Red	BN01021-4
HCHO Standard (100 mM)	100 µl	Yellow	BN01021-5

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plate is preferred for this assay.
- Multi-well fluorescence spectrophotometer.
- 10 kDa Spin Column

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **HCHO Assay Buffer**: Bring to room temperature before use. Store at -20°C. Stable for two months.
- **Formaldehyde Assay Kit:** Ready to use as supplied. Warm to room temperature before use. Store at -20°C.
- **HCHO Enzyme Mix:** Reconstitute with 220 µl HCHO Assay Buffer. Pipette gently to dissolve. Store at -20°C. Keep on ice while in use. Stable for two months.
- **HCHO Developer:** Reconstitute with 220 µl HCHO Assay Buffer. Pipette gently to dissolve. Aliquot & store at -20°C. Keep on ice while in use. Stable for two months.
- **HCHO Standard:** Ready to use as supplied. Bring Standard to room temperature before use. Store at -20°C.

VII. Formaldehyde Assay Protocol:

1. **Sample Preparation:** Centrifuge biological fluids (i.e. saliva, serum, plasma, urine) at 10,000 X g for 5 min. at 4°C. Collect supernatant & add 1-50 µl into desired well(s) in a 96-well plate. Adjust the volume to 50 µl/well with HCHO Assay Buffer. Homogenize tissue (~10 mg) or cells (2 x 10^6) on ice using 100 µl of HCHO Assay Buffer. Centrifuge the homogenate at 10,000 X g for 10 min. at 4°C. Collect supernatant and add 1-50 µl into desired well(s) in a 96-well plate. Adjust the volume to 50 µl/well with HCHO Assay Buffer.

   Notes:

   a. Formaldehyde concentrations can vary over a wide range. We recommend doing a pilot experiment using several doses to ensure the readings are within the Standard Curve range.

   b. Endogenous compounds in the sample may contribute to the background signal. If high interference is predicted in the sample, prepare parallel sample well(s) as sample background control(s).

   c. For samples having high-protein content, we recommend deproteinizing the samples (tissue lysate or biological fluids) using 10 kDa Spin Column. Add sample to the spin column, centrifuge at 10,000 X g for 10 min. at 4°C. Collect the filtrate.

   d. For urine samples, it is recommended to dilute 10-20X using HCHO Assay Buffer. Mix well & add 5-50 µl of the diluted sample into desired well(s) in a 96-well plate. Adjust the volume to 50 µl/well with HCHO Assay Buffer.

   e. To ensure accurate determination of HCHO in the test samples or for samples having low concentrations of HCHO, we recommend spiking samples with a known amount of HCHO Standard (300 pmol).

2. **Standard Curve Preparation:** Prepare 1 mM HCHO Standard by adding 10 µl of 100 mM HCHO Standard into 990 µl of ddH_2O. Dilute further to 50 µM by adding 50 µl of 1 mM Standard into 950 µl of ddH_2O. Add 0, 2, 4, 6, 8, and 10 µl of diluted 50 µM HCHO Standard

into a series of wells in a 96-well plate to generate 0, 100, 200, 300, 400 & 500 pmol/well of HCHO Standard. Adjust the volume to 50 µl/well with HCHO Assay Buffer.

Note: Always prepare fresh diluted HCHO Standard solution & use within 4 hrs.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total of 50 µl of reaction mix.

	Reaction Mix	* Background Mix
HCHO Assay Buffer	43 µl	45 µl
HCHO Enzyme Mix	2 µl	---
HCHO Developer	2 µl	2 µl
PicoProbe™	3 µl	3 µl

Mix well. Add 50 µl of the Reaction Mix to each well containing Standards and samples. Mix well.

* For samples having background, add 50 µl of Background Mix to background control well(s).

4. **Measurement:** Incubate the plate at 25°C for 20 min., protected from light. Measure fluorescence (Ex/Em = 535/587 nm) in a plate reader.

5. **Calculation:** Subtract 0 HCHO Standard reading from all readings. Plot the HCHO Standard Curve. If sample background control reading is significant, subtract background control reading from sample reading. Apply the corrected RFU to the HCHO Standard Curve to get B pmol of HCHO in the sample well.

Sample HCHO concentration (C) = B/V X D pmol/µl or µM

Where: **B** is the amount of HCHO in the sample well from Standard Curve (pmol)
V is the sample volume added into the reaction well (µl)
D is the sample dilution factor

Note: For spiked samples, correct for any sample interference by using following equation:

$$\textbf{HCHO concentration in spiked sample well (B)} = \left(\frac{(\mathrm{RFU}_{\text{sample (corrected)}})}{(\mathrm{RFU}_{\text{sample + HCHO Std(corrected)}})\text{-}(\mathrm{RFU}_{\text{sample(corrected)}})}\right) * HCHO\ Spike\ (\mathrm{pmol})$$

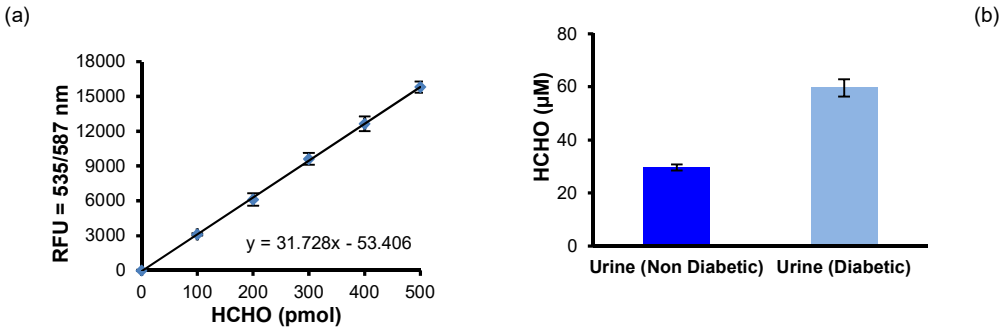


Figure: (a) HCHO Standard Curve. (b) Measurement of Formaldehyde concentration in human urine from non-diabetic and diabetic donors. Samples were deproteinized using 10 kDa Spin Column and diluted 10X. Diluted samples (20 µl) were spiked with known amount of HCHO (300 pmol). Sample preparation and assay was performed following kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.