

# **Formaldehyde Assay Kit**

Item No. 700380

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## GENERAL INFORMATION

### Materials Supplied

Item Number	Item	Quantity/Size
700381	Formaldehyde Detector	1 vial/150 mg
700382	Formaldehyde Ammonium Acetate	1 vial/5 ml
700383	Formaldehyde DMSO	1 vial/2 ml
700384	Formaldehyde Standard	1 vial/100 µl
400091	Half Volume 96-Well Solid Plate (black)	1 plate
400012	96-Well Cover Sheet	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.



**WARNING:** This product is for laboratory research use only; not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

## Precautions

Please read these instructions carefully before beginning this assay.

**Formaldehyde is carcinogenic. It is toxic if inhaled, ingested, or if in contact with skin. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes. Keep away from combustible materials.**

**For research use only. Not for human or diagnostic use.**

## If You Have Problems

### Technical Service Contact Information

**Phone:** 888-526-5351 (USA and Canada only) or 734-975-3888

**Fax:** 734-971-3641

**Email:** techserv@caymanchem.com

**Hours:** M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if stored at room temperature and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. A fluorometer with the capacity to measure fluorescence using excitation wavelength between 365-375 nm and emission wavelength between 465-475 nm
2. Adjustable pipettes and a repeat pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

## INTRODUCTION

### Background

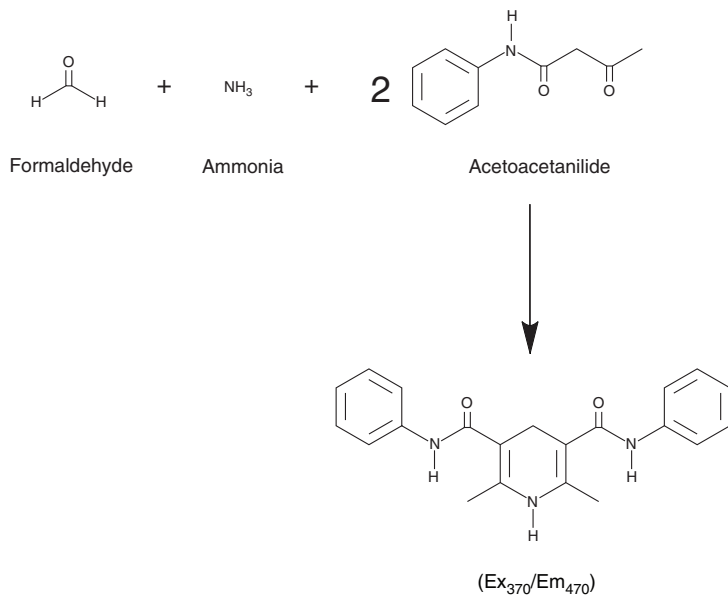
Formaldehyde (CH<sub>2</sub>O, methanal) is an important industrial compound with numerous applications ranging from production of resins and building materials to medicine. At room temperature, it is a flammable and colorless gas with a strong pungent odor. It is a naturally occurring biological compound present in all cells, tissues, and body fluids. The biochemical pathway of formaldehyde production *via* the demethylation/methylation process is generally connected to the methionine-homocysteine cycle.<sup>1-3</sup> Formaldehyde is also generated during microsomal cytochrome P450-dependent oxidation of xenobiotics.<sup>1</sup> It is also a product of oxidative deamination of methylamine catalyzed by semicarbazide-sensitive amine oxidase (SSAO).<sup>4</sup> Increased SSAO-mediated deamination has been implicated in some pathophysiological conditions, such as diabetic complications.<sup>4</sup>

Formaldehyde can also be released by burning wood, kerosene, natural gas, or cigarettes, and is present in automobile emissions. However, the highest level of human exposure to this aldehyde occurs in occupational settings like industrial workers, embalmers, and pathology anatomists resulting in an increased risk for leukemia and nasopharyngeal cancers compared to the general population.<sup>5,6</sup>

Quantification of formaldehyde in urine was recently shown to be a promising tool in the investigation of cancer, because formaldehyde is elevated in certain cancer cell types.<sup>7,8</sup> An increased concentration of formaldehyde in urine has been associated with prostate and bladder cancer and an elevated level of formaldehyde in breath has been associated with breast cancer.<sup>7-10</sup>

### About This Assay

Cayman's Formaldehyde Assay provides a convenient fluorescence-based method for detecting formaldehyde in urine. The cyclization between formaldehyde and acetoacetanilide in the presence of ammonia results in a fluorescent product which is analyzed using an excitation wavelength between 365-375 nm and an emission wavelength between 465-475 nm (see Figure 1, on page 6).<sup>11</sup>



**Figure 1. Assay scheme**

## PRE-ASSAY PREPARATION

### Reagent Preparation

#### 1. Formaldehyde Detector - (Item No. 700381)

The vial contains 150 mg of acetoacetanilide. To prepare for use in the assay, weigh 42 mg into another vial, add 200  $\mu$ l of Formaldehyde DMSO (Item No. 700383), and vortex until dissolved. Add 200  $\mu$ l of HPLC-grade water to the vial and vortex. The reagent is now ready to use in the assay. This is enough detector to assay 40 wells. Prepare additional Detector if assaying more wells. The reconstituted Detector is stable for eight hours at room temperature. If Detector crystallizes out of solution, gently warm and vortex until it goes back into solution.

#### 2. Formaldehyde Ammonium Acetate - (Item No. 700382)

The vial contains 5 ml of ammonium acetate. The reagent is ready to use in the assay.

#### 3. Formaldehyde DMSO - (Item No. 700383)

The vial contains 2 ml of dimethylsulfoxide (DMSO). It is ready to use to dissolve the Formaldehyde Detector.

#### 4. Formaldehyde Standard - (Item No. 700384)

The vial contains 100  $\mu$ l of 3 M formaldehyde. It is ready to use to prepare the standard curve. *NOTE: Do not store Formaldehyde below 4°C or it may polymerize.*

## Sample Preparation

### Urine

Typically, human urine has a formaldehyde concentration in the range of 40-1,400  $\mu\text{M}$ .<sup>12</sup>

1. Collection of urine does not require any special treatment.
2. If urine samples contain visible particulates, then the sample can be either filtered or centrifuged at 1,000-2,000 x g for 10 minutes.
3. If not assaying the same day, urine can be stored at 4°C overnight or at -80°C for long term storage. The urine sample will be stable for one month at -80°C.
4. Urine samples should be dilute 1:2-1:5 with HPLC-grade water before assaying.

*NOTE: Formaldehyde values from urine samples can be standardized using Cayman's Creatinine (urinary) Assay Kit (Item No. 500701).*

## ASSAY PROTOCOL

### Plate Set Up

There is no specific pattern for using the wells on the plate. However, a formaldehyde standard curve in duplicate has to be assayed with the samples. We suggest that each sample be assayed at least in duplicate and to have two wells designated as sample background wells to allow for the correction of non-formaldehyde-generated fluorescence. Record the contents of each well on the template sheet provided on page 19. A typical layout of samples to be measured in duplicate is given below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	(A)	(A)	(S1)	(S1)	(S5)	(S5)	(S9)	(S9)	(S13)	(S13)	(S17)	(S17)
B	(B)	(B)	(B1)	(B1)	(B5)	(B5)	(B9)	(B9)	(B13)	(B13)	(B17)	(B17)
C	(C)	(C)	(S2)	(S2)	(S6)	(S6)	(S10)	(S10)	(S14)	(S14)	(S18)	(S18)
D	(D)	(D)	(B2)	(B2)	(B6)	(B6)	(B10)	(B10)	(B14)	(B14)	(B18)	(B18)
E	(E)	(E)	(S3)	(S3)	(S7)	(S7)	(S11)	(S11)	(S15)	(S15)	(S19)	(S19)
F	(F)	(F)	(B3)	(B3)	(B7)	(B7)	(B11)	(B11)	(B15)	(B15)	(B19)	(B19)
G	(G)	(G)	(S4)	(S4)	(S8)	(S8)	(S12)	(S12)	(S16)	(S16)	(S20)	(S20)
H	(H)	(H)	(B4)	(B4)	(B8)	(B8)	(B12)	(B12)	(B16)	(B16)	(B20)	(B20)

A-H = Standards

S1-S20 = Sample Wells

B1-B20 = Background Sample Wells

**Figure 2. Sample plate format**

### Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

### General Information

- The final volume of the assay is 100  $\mu\text{l}$  in all the wells.
- It is not necessary to use all the wells on the plate at one time.
- We recommend assaying samples in triplicate, but it is the user's discretion to do so.
- The assay is performed at room temperature.
- Monitor the fluorescence with an excitation wavelength between 365-375 nm and emission wavelength between 465-475 nm.

### Standard Preparation

Dilute 10  $\mu\text{l}$  of the Formaldehyde Standard with 9.99 ml of HPLC-grade water to yield a concentration of 3 mM. Dilute 200  $\mu\text{l}$  of the 3 mM Formaldehyde with 1.8 ml of HPLC-grade water to yield the stock solution of 300  $\mu\text{M}$ . The 300  $\mu\text{M}$  Standard will be used to prepare the standards. Take eight clean glass test tubes and mark them A-H. Add the amount of Formaldehyde Standard (300  $\mu\text{M}$ ) and HPLC-grade water to each tube as described in Table 1. The diluted standards are stable for four hours at room temperature.

Tube	Formaldehyde 300 $\mu\text{M}$ Standard ( $\mu\text{l}$ )	HPLC-grade water ( $\mu\text{l}$ )	Final Concentration ( $\mu\text{M}$ )
A	0	1,000	0
B	10	990	3
C	20	980	6
D	60	940	18
E	100	900	30
F	200	800	60
G	400	600	120
H	500	500	150

**Table 1. Formaldehyde standards**

## Performing the Assay

1. **Standard Wells** - add 50  $\mu\text{l}$  of standard (tubes A-H) and 40  $\mu\text{l}$  of Ammonium Acetate per well in the designated wells on the plate (see Figure 2, page 9).
2. **Sample Wells** - add 50  $\mu\text{l}$  of sample and 40  $\mu\text{l}$  of Ammonium Acetate to two wells. When necessary, samples should be diluted with HPLC-grade water to fall within the range of the assay.
3. **Sample Background Wells** - add 10  $\mu\text{l}$  of HPLC-grade water, 50  $\mu\text{l}$  of sample, and 40  $\mu\text{l}$  of Ammonium Acetate to two wells.
4. Initiate the reactions by adding 10  $\mu\text{l}$  of Formaldehyde Detector to the standard and sample wells only. **Do not** add the Detector to the sample background wells.
5. Cover the plate with the plate cover and incubate for 15 minutes at room temperature.
6. Remove the plate cover and read the fluorescence using an excitation wavelength between 365-375 nm and emission wavelength between 465-475 nm.

## ANALYSIS

### Calculations

1. Determine the average fluorescence of each sample and sample background wells.
2. Subtract the fluorescence of the sample background wells from the fluorescence of the sample wells to yield the corrected sample fluorescence (CSF).
3. Determine the average fluorescence of the standards. Subtract the fluorescence value of the standard A from itself and all other standards. This is the corrected fluorescence.
4. Plot the corrected fluorescence values (from step 3 above) of each standard as a function of the final concentration of formaldehyde from Table 1. See Figure 3, on page 14, for an example of a typical standard curve (do not use this example to calculate concentrations of formaldehyde in your samples).
5. Calculate the formaldehyde concentration of the samples using the equation obtained from the linear regression of the standard curve substituting the corrected sample fluorescence (CSF) for each sample.

$$\text{Formaldehyde } (\mu\text{M}) = \left[ \frac{\text{CSF} - (\text{y-intercept})}{\text{Slope}} \right] \times \text{Sample dilution}$$

*NOTE: Formaldehyde values from urine samples can be standardized using Cayman's Creatinine (urinary) Assay Kit (Item No. 500701).*

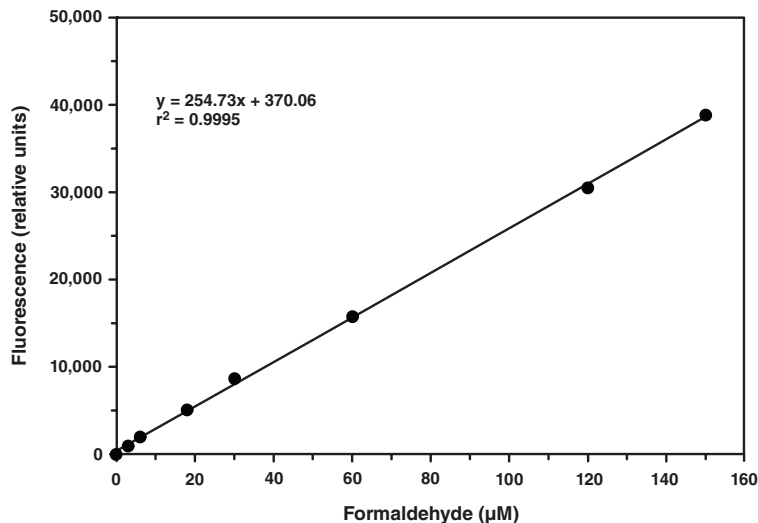


Figure 3. Example of a formaldehyde standard curve

## Performance Characteristics

### Precision:

When a series of 16 human urine measurements were performed on the same day, the intra-assay coefficient of variation was 2.5%. When a series of 16 human urine measurements were performed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 3.2%.

### Assay Range:

Under the standardized conditions of the assay described in this booklet, the dynamic range of the kit is 0-150 µM of formaldehyde.

## RESOURCES

### Interferences

The following reagents were tested for their ability to give a false reading in the assay. Compounds were tested in the presence and absence of 30 µM formaldehyde.

Compound	% Interference
Acetaldehyde (500 µM)	<0.01%
Propionaldehyde (500 µM)	<0.01%
Acetone (1 mM)	<0.01%
Methanol (3 mM)	<0.001%
Magnesium Chloride (1 mM)	<0.001%
Sodium Chloride (25 mM)	<0.001%
Potassium Chloride (5 mM)	<0.001%
Calcium Chloride (100 µM)	<0.01%
β-hydroxybutyrate (500 µM)	<0.001%



## Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No fluorescence was detected above background in the sample wells	Sample was too dilute	Re-assay the sample using a lower dilution
Fluorometer exhibited 'MAX' values for the wells	The GAIN setting is too high	Reduce the GAIN and re-read
Sample fluorescence was higher than the last standard	Sample is too concentrated	Dilute the sample and re-assay with HPLC-grade water

## References

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## Related Products

Aldehyde Site (DNA and Protein) Detection Kit - Item No. 600170

Creatinine Assay Kit - Item No. 500701

Demethylase (Jumonji-type) Activity Assay Kit - Item No. 700390

Demethylase (LSD-type) Activity Assay Kit - Item No. 700400

DNA Methylation EIA Kit - Item No. 589324

JMJD2A Inhibitor Screening Assay Kit - Item No. 700360

JMJD2D Inhibitor Screening Assay Kit - Item No. 700370

LSD1 Inhibitor Screening Assay Kit - Item No. 700120

Methyltransferase Colorimetric Assay Kit - Item No. 700140

Methyltransferase Fluorometric Assay Kit - Item No. 700150

SET7/9 Methyltransferase Inhibitor Screening Assay Kit - Item No. 700270

SET8 Methyltransferase Inhibitor Screening Assay Kit - Item No. 700350

## Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

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