

ARBOR ASSAYS™  
Interactive Assay Solutions™



# DetectX®

## Formaldehyde Fluorescent Detection Kit

2 Plate Kit Catalog Number K001-F1

Species Independent

### Sample Types Validated:

**Human Urine and Tissue Culture Media**

*Covered under US Patent numbers 8,173,386 & 8,765,396*

Please read this insert completely prior to using the product.  
For research use only. Not for use in diagnostic procedures.

[www.ArborAssays.com](http://www.ArborAssays.com)   

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## BACKGROUND

Formaldehyde (methanal),  $\text{H}_2\text{C}=\text{O}$ , is a colorless, flammable, strong-smelling gas. It is an important industrial chemical used to manufacture building materials and to produce many household products. In the US approximately  $3 \times 10^9$  kg are produced annually<sup>1</sup>. In addition, formaldehyde is commonly used as an industrial fungicide, germicide, and disinfectant, and as a preservative in mortuaries and medical laboratories. Materials containing formaldehyde can release formaldehyde gas or vapor into the air. Formaldehyde can also be released by burning wood, kerosene, natural gas, or cigarettes, from automobile emissions, and from natural processes.

Formaldehyde can undergo rapid chemical changes immediately after absorption. Studies have suggested that formaldehyde may affect the lymphatic and blood systems and that exposure to formaldehyde may cause leukemia, particularly myeloid leukemia, in humans.

Industrial workers who help to produce formaldehyde or formaldehyde-containing products, laboratory technicians, health care professionals, and mortuary employees may be exposed to higher levels of formaldehyde than the general public<sup>2</sup>. Exposure occurs primarily by inhaling formaldehyde gas or vapor from the air or by absorbing liquids containing formaldehyde through the skin. The National Cancer Institute (NCI) has determined that there is an association between occupational exposure to formaldehyde and an increase in the risk of cancer. Several NCI studies have found that anatomists and embalmers, professions with potential exposure to formaldehyde, are at an increased risk for leukemia and brain cancer compared with the general population. For example a multi-centered US study determined increased risk of nasopharyngeal cancer with formaldehyde exposure<sup>3</sup>.

1. US Consumer Product Safety Commission, Release #79-059.
2. International Agency for Research on Cancer, June 2004, [www.iarc.fr/ENG/Press\\_Releases/archives/pr153a.html](http://www.iarc.fr/ENG/Press_Releases/archives/pr153a.html)
3. TL Vaughan, et al, Occup. Environ. Med., 2000, 57, 376-384.

## ASSAY PRINCIPLE

The DetectX® Formaldehyde Kit is designed to quantitatively measure formaldehyde present in tissue culture media and urine samples. Please read the complete kit insert before performing this assay. A formaldehyde standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a black microtiter plate. The fluorescent reaction is initiated with the DetectX® Formaldehyde reagent, which is pipetted into each well. After a short incubation the emission of the generated fluorescent signal is detected in a microtiter plate reader capable of measuring 510 nm fluorescence utilizing 450 nm excitation wavelength. The concentration of the formaldehyde in the sample is calculated, after making a suitable correction for the dilution of the sample, using software available with most fluorescence plate readers.

## RELATED PRODUCTS

Kits	Catalog No.
Urinary Creatinine Detection Kit (2 or 10 Plate)	K002-H1/H5

## SUPPLIED COMPONENTS

### Black Half Area 96 Well Plate

See: [www.ArborAssays.com/resources/#general-info](http://www.ArborAssays.com/resources/#general-info) for plate dimension data.

2 plates

Catalog Number X037-2EA

### Formaldehyde Standard

2,000  $\mu$ M formaldehyde solution in special stabilizing solution. Outer container has formaldehyde absorbing pad. The standard is stable if kept tightly sealed. **KEEP TIGHTLY SEALED.**

500  $\mu$ L

Catalog Number C001-500UL

### DetectX<sup>®</sup> Formaldehyde Reagent

Special formulation of reagents to detect formaldehyde in solution. Contains 0.09% sodium azide as a preservative.

5 mL

Catalog Number C002-5ML

### Plate Sealers

2 each

Catalog Number X002-1EA

## STORAGE INSTRUCTIONS

All components of this kit should be stored at 4°C until the expiration date of the kit.

## OTHER MATERIALS REQUIRED

A supply of distilled or deionized water free of formaldehyde.

Repeater pipet with disposable tips capable of dispensing 25  $\mu$ L.

An incubator capable of accurately maintaining 37°C.

Fluorescence 96 well plate reader capable of reading fluorescent emission at 510 nm, with excitation at 450 nm. Set plate parameters for a 96-well Corning Costar 3694 plate.

See [www.ArborAssays.com/resources/#general-info](http://www.ArborAssays.com/resources/#general-info) for plate dimension data.

Software for converting raw relative fluorescent unit (FLU) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

**It should be noted that most reactions should be compatible with the formaldehyde readout system. In systems where the amount of formaldehyde produced is low the amount of generated fluorescence will also be low. Only plate readers that are capable of measuring dim fluorescent signals and having adjustable gain or filter settings may be compatible.**

## PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Formaldehyde is a toxic, volatile, reactive chemical that can form adducts with proteins and nucleic acids. It reacts with oxygen to form formic acid and so should be kept sealed and only used in well-ventilated laboratories. For disposal, we suggest discarding all excess standards and samples in a 10% aqueous solution of sodium bisulfite, such as Sigma catalog number 13438.

Some of the components of this kit contain sodium azide as a preservative, which may react with lead or copper plumbing to form potentially explosive complexes. When disposing of reagents always flush with large volumes of water to prevent azide build-up.

## SAMPLE TYPES

Formaldehyde is identical across all species and cell types.

Urine and most types of tissue culture media (TCM) are compatible with this assay.

## SAMPLE PREPARATION

Urine samples containing visible protein or particulates should be centrifuged or filtered prior to using. Urine samples must be diluted 1:4 with water by taking one part of sample and adding 3 parts of water prior to using in the kit. Any urine samples with formaldehyde concentrations outside the standard curve range should be diluted further with water to obtain readings within the standard curve.

TCM samples should be diluted in TCM and read off a standard curve generated in the same TCM.

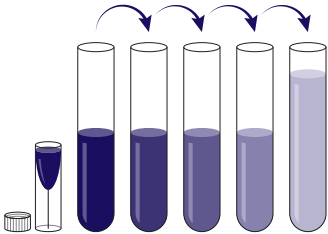
**Use all diluted samples within 2 hours of preparation.**

# REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

## Standard Preparation

Label glass test tubes as #1 through #7. Pipet 450  $\mu\text{L}$  of water into tube #1 and 250  $\mu\text{L}$  into tubes #2-#7. Add 50  $\mu\text{L}$  of the Formaldehyde stock solution to tube #1 and vortex completely. Take 250  $\mu\text{L}$  of the formaldehyde solution in tube #1 and add it to tube #2 and vortex completely. Add 250  $\mu\text{L}$  of tube #2 to tube #3 and vortex completely. Repeat this serial dilution for tubes #4 through #7. The concentration of formaldehyde in tubes 1 through 7 will be 200, 100, 50, 25, 12.5, 6.25 and 3.125  $\mu\text{M}$ . Water will be used as a sample blank.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Water Volume ( $\mu\text{L}$ )	450	250	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition ( $\mu\text{L}$ )	50	250	250	250	250	250	250
Final Conc ( $\mu\text{M}$ )	200	100	50	25	12.5	6.25	3.125



## ASSAY PROTOCOL

**We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine formaldehyde concentrations.**

1. A plate layout sheet has been included on the back page of the insert to aid proper sample and standard identification. Set plate parameters for a 96-well Corning Costar 3694 plate. See [www.ArborAssays.com/resources/#general-info](http://www.ArborAssays.com/resources/#general-info) for plate dimension data.
2. Pipet 50  $\mu$ L of samples, water as the blank or standards into wells in the black plate.
3. Add 25  $\mu$ L of the DetectX<sup>®</sup> Formaldehyde Reagent to each well using a repeater pipet.
4. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and press to seal adequately.
5. Incubate at 37°C for 30 minutes. Room temperature incubation will yield approximately 75% of the fluorescent signal generated with 37°C incubation.
6. Read the fluorescent signal from each well in a plate reader capable of reading the fluorescent signal at 510 nm with excitation at 450 nm. Please contact your plate reader manufacturer for suitable filter sets.
7. Use the plate reader's built-in 4PLC software capabilities to calculate formaldehyde concentrations for each sample.

## CALCULATION OF RESULTS

Average the duplicate FLU readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean FLUs for the zero standard. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:

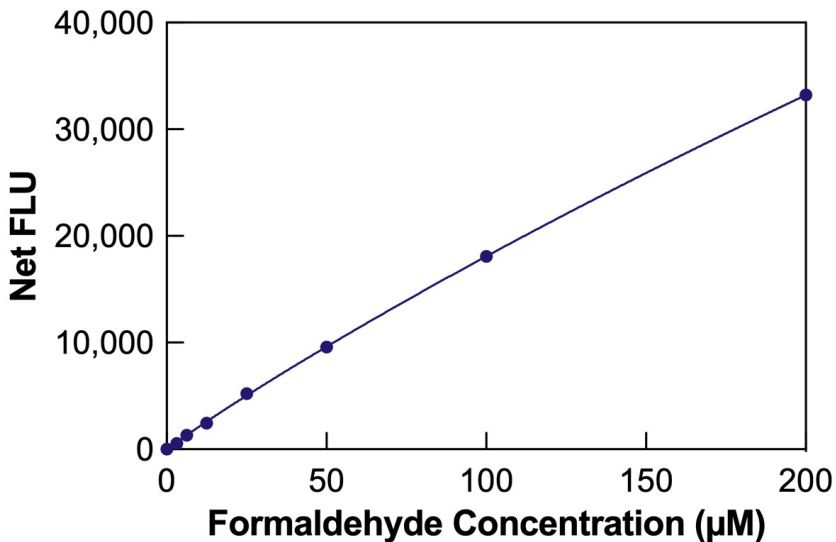
[www.myassays.com/arbor-assays-formaldehyde-detection-kit.assay](http://www.myassays.com/arbor-assays-formaldehyde-detection-kit.assay)

TYPICAL DATA

Sample	Mean FLU	Net FLU	Formaldehyde Conc. (µM)
Zero	786	--	0
Standard 1	33,981	33,203	200
Standard 2	18,844	18,067	100
Standard 3	10,347	9,569	50
Standard 4	6,001	5,224	25
Standard 5	3,228	2,451	12.5
Standard 6	2,104	1,326	6.25
Standard 7	1,334	557	3.125
Sample 1	18,387	17,610	97.1
Sample 2	3,599	2,821	13.8

Always run your own standard curve for calculation of results. Do not use this data.

## Typical Standard Curve



Always run your own standard curve for calculation of results. Do not use this data.

## VALIDATION DATA

### Sensitivity and Limit of Detection

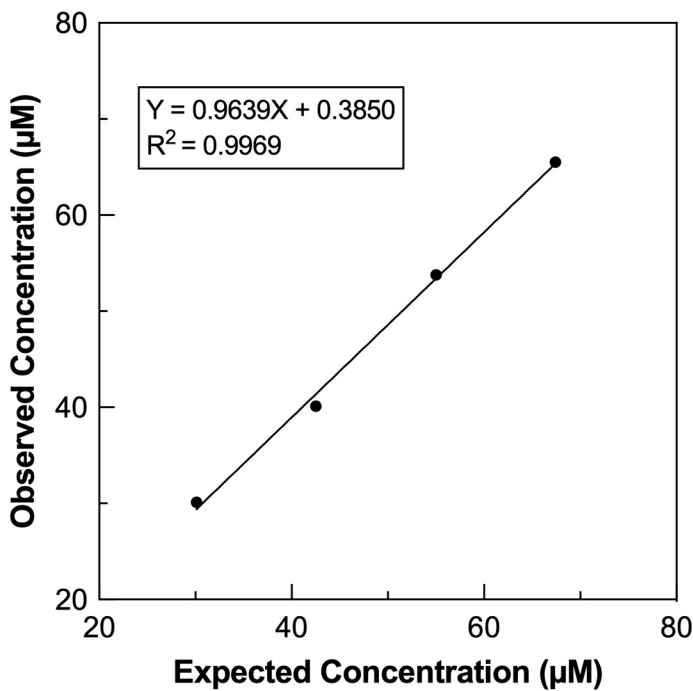
Sensitivity was calculated by comparing the FLU's for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the Zero along the standard curve.

**Sensitivity was determined as 0.715 µM**

Linearity

Linearity was determined by taking two human urine samples, one with a low formaldehyde level of 17.6 µM and one with a higher level of 79.9 µM, and mixing them in the ratios given below. The measured concentrations were compared to the expected values.

Low Urine	High Urine	Observed Conc. (µM)	Expected Conc. (µM)	% Recovery
80%	20%	30.1	30.1	100.1
60%	40%	40.1	42.5	94.3
40%	60%	53.8	55.0	97.9
20%	80%	65.5	67.4	97.1
Mean Recovery				97.4%



### Intra Assay Precision

Four human urine samples were diluted 1:4 with deionized water and run in replicates of 20 in an assay. The mean and precision of the calculated formaldehyde concentrations were:

Sample	Formaldehyde Conc. (µM)	%CV
1	9.70	7.3
2	38.3	4.2
3	76.0	3.4
4	162	3.7

### Inter Assay Precision

Four human urine samples were diluted 1:4 with deionized water and run in duplicates in 20 assays run over two days by two operators. The mean and precision of the calculated formaldehyde concentrations were:

Sample	Formaldehyde Conc. (µM)	%CV
1	10.5	6.7
2	36.9	4.5
3	71.1	3.8
4	148.9	4.3

## SAMPLE VALUES

Eighteen random clean catch urine samples were run in the assay. Formaldehyde concentrations in the neat urine ranged from 18 to 776  $\mu\text{M}$  with an average of 225  $\mu\text{M}$ . These samples were also run in the DetectX<sup>®</sup> Urinary Creatinine Detection kit, K002-H1/H5, and the formaldehyde levels normalized to creatinine concentration. Normalized values ranged from 73.0 to 1,026  $\mu\text{moles formaldehyde/gram creatinine}$ .

## CROSS REACTIVITY

A variety of aldehydes, ketones and inorganic compounds were tested for their ability to give a false reading in the assay. These were made up at 0.1M (equal to 100,000  $\mu\text{M}$ ), diluted to 100  $\mu\text{M}$  and tested in the assay. The following cross reactivities were observed.

Compound	% Cross Reactivity
Acetone	<0.01%
Propionaldehyde	<0.01%
Acetaldehyde	<0.02%
Magnesium Chloride	0.01%
Methanol	<0.001%
Sodium Chloride	<0.001%

## INTERFERENTS

A variety of inorganic compounds were tested for their ability to give a false negative reading in the assay by reacting with the formaldehyde present in the sample. For example, sodium bisulfite,  $\text{Na}_2\text{SO}_3$ , is a molecule that reacts with aldehydes and ketones to form bisulfite addition compounds. The aldehyde addition compound/formaldehyde mixtures would therefore have little or no free formaldehyde present in them. These were made up at 0.1M (equal to 100,000  $\mu\text{M}$ ) and diluted to below 1  $\mu\text{M}$  and tested in the assay. They were also added to samples containing a known amount of formaldehyde to show that they were reacting with formaldehyde. The following is a list of the known interferants and their lower levels of interference in the reaction.

Compound	Known Reaction Limit
Copper(II) Chloride	>1,000 $\mu\text{M}$
Copper(III) Chloride	>1 $\mu\text{M}$
Iron(III) Chloride	>1 $\mu\text{M}$
Iron(II) Sulfate	>1 $\mu\text{M}$
Sodium Bisulfite	>1 $\mu\text{M}$

## LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

## CONTACT INFORMATION

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## OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with EIA kits for wildlife conservation research.

*DetectX<sup>®</sup>, ThioStar<sup>®</sup> and the Arbor Assays logo are all registered trademarks.*

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B												
C												
D												
E												
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