DetectX® Formaldehyde

Fluorescent Detection Kit

2 Plate Kit – Catalog No. K001-F1

Species Independent

Sample Types Tested:

Human Urine and Tissue Culture Media (TCM)

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.
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SUPPLIED COMPONENTS & STORAGE

		K001-F1	Description
Black Half Area 96 Well Plate	Quantity	2	Corning Costar 3694 plate
	Catalog No.	X037-2EA	
Formaldehyde Standard	Volume	500 µL	2,000 µM Formaldehyde in stabilizing solution. Outer container has a Formaldehyde absorbing pad. **KEEP TIGHTLY SEALED.**
	Catalog No.	C001-500UL	
DetectX® Formaldehyde Reagent	Volume	5 mL	Formaldehyde detection reagent in solution. Contains 0.09% sodium azide as a preservative.
	Catalog No.	C002-5ML	
Plate Sealer	Quantity	2	-
	Catalog No.	X002-1EA	

This kit should be stored at 4°C up to the expiration date on the kit label.

OTHER MATERIALS REQUIRED

- Distilled or deionized water
- Adjustable pipettes with disposable tips. Repeater pipettes or multichannel pipettes with corresponding tips are also recommended.
- Glass test tubes for Standard and Sample preparation. **Do not use plastic**.
- An incubator set to 37°C
- A fluorescence plate reader capable of reading fluorescent emission at 510 nm, with excitation at 450 nm.
- Software for converting raw relative fluorescent unit (FLU) readings from the plate reader and carrying out four parameter logistic curve (4PL) fitting. Contact your plate reader manufacturer for details.
    - **Note:** In systems where the amount of formaldehyde produced is low the amount of generated fluorescence will also be low. In these cases, only plate readers capable of measuring dim fluorescent signals and that have adjustable gain or filter settings may be compatible.

PRECAUTIONS

- Read this insert completely prior to using the product.
- This kit may not perform as described if any reagent or procedure is replaced or modified. Do not interchange reagents from different kit lots.
- Take appropriate safety precautions, such as: avoid breathing fumes, wear personal protective equipment (gloves, clothing, eye and face protection), and familiarize yourself with SDS documents.
    - https://www.arborassays.com/documentation/msds/K001-F1_MSDS.pdf
- 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, discard 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.

BACKGROUND

Formaldehyde (methanal), $H_2C=O$, 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 United States approximately 3×10^9 kg are produced annually.[1] 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 through 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.[2] 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.[3]

ASSAY PRINCIPLE

The DetectX® Formaldehyde Detection 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 added into a black microtiter plate. The fluorescent reaction is initiated upon addition of the DetectX® Formaldehyde Reagent to the plate. 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.

Formaldehyde is identical across all species and cell types.

REAGENT PREPARATION

Allow all kit reagents to come to room temperature for 30 minutes. Room temperature for this assay is defined as 22°C – 24°C.

SAMPLE PREPARATION

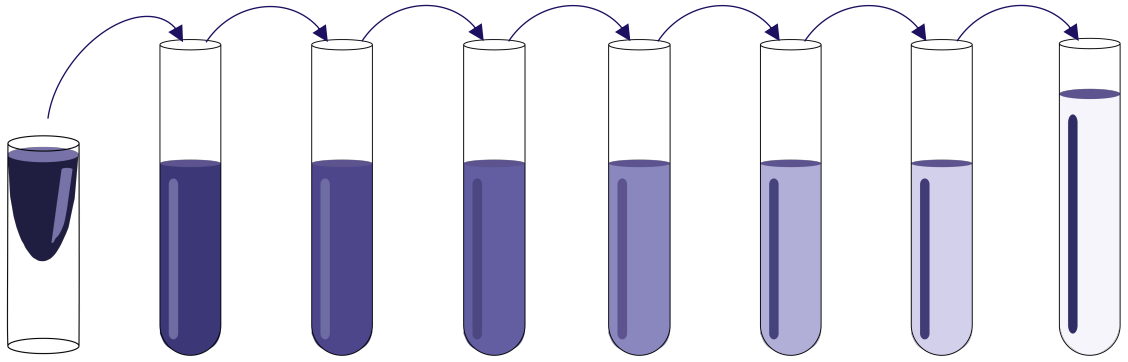
Sample Type	Procedure
Urine	• Urine samples containing visible protein or particulates should be centrifuged or filtered prior to using. • Urine samples must be diluted 1:4 with water by combining one part of sample and adding 3 parts of water prior to using in the kit. • Any urine samples with Formaldehyde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

Tissue Culture Media (TCM)	• TCM samples should be diluted in TCM and read off a standard curve generated in the same TCM.
	• Checking for interference prior to committing samples:
	  ○ Compare a Standard curve diluted in water to a Standard curve diluted in TCM.
	  ○ Prepare a dose response for any cell treatments.

⚠ **Use all diluted Samples within 2 hours of preparation.**

STANDARD PREPARATION

1. Label glass tubes Standard 1 through Standard 7.
2. Add 450 µL of water to Standard 1 tube.
3. Add 250 µL of water to Standard 2 – 7 tubes.
4. Briefly vortex the Formaldehyde Standard stock and add 50 µL to Standard 1 tube. Vortex thoroughly.
5. Transfer 250 µL of Standard 1 into Standard2 tube. Vortex thoroughly.
6. Transfer 250 µL of the mixed solution from Standard 2 into Standard 3. Vortex thoroughly.
7. Continue serially diluting into the remaining tubes. This process and the final concentrations are summarized in the table below.



	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
Water Volume (µL)	450	250	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	50	250	250	250	250	250	250
Final Concentration (µM)	200	100	50	25	12.5	6.25	3.13

⚠ **Use all Standards within 2 hours of dilution.**

ASSAY PROTOCOL

Before You Begin:

- **Room Temperature for this assay is defined as 22ºC – 24ºC.**
- **Ensure all reagents have been warmed to room temperature.**
- **Dilute Samples as described in Sample Preparation.**
- **Run all Standards and Samples in duplicate.**
- Use the blank plate template on the back page of this booklet to design your plate layout and aid in proper sample and standard identification.
- Set plate parameters for a 96-well Corning Costar 3694 plate. See ArborAssays.com for plate dimension data.

1. Add 50 µL of Samples, Standards, or water (Zero Standard) into duplicate wells.
2. Add 25 µL of the DetectX® Formaldehyde Reagent to each well. Gently tap the sides of the plate to ensure adequate mixing.
3. Cover the plate with the plate sealer and incubate at **37ºC for 30 minutes**.
4. After the incubation, read the plate immediately using fluorescent emission at 510 nm with excitation at 450 nm.

CALCULATION OF RESULTS

Follow the instructions below or use this online tool: https://myassays.com/assay.aspx?id=931

1. Use four-parameter logistic curve (4PL) software to calculate the Formaldehyde concentration for each sample. Gather all raw data fluorescence (FLU) readings from each Sample and Standard, including the Zero Standard.
2. Average the duplicate FLU readings for each Sample, Standard, and Zero Standard (Mean FLU).

EXAMPLE:

Sample	Replicate 1 FLU	Replicate 2 FLU	Mean FLU
Zero Standard	776	796	786
Standard 1	33,961	34,001	33,981
Sample 1	18,382	18,392	18,387

3. Subtract the Zero Standard Mean FLU from the Mean FLU for each Sample and Standard (Net FLU).

EXAMPLE:

Sample	Mean FLU	Zero Standard Mean FLU	Net FLU
Standard 1	33,981	786	33,195
Sample 1	18,387	786	17,601

4. Plot the standard curve with Net FLU for the Standards on the y-axis and Formaldehyde concentration (µM) on the x-axis. Perform a 4PL fit.

   Use the sample Net FLU readings and the 4PL fit to calculate the Formaldehyde concentration in diluted samples. If diluted sample Formaldehyde concentration is outside of the range of the standards, the sample should be prepared again at a more appropriate dilution.

EXAMPLE:

Sample	Net FLU	Formaldehyde Concentration (µM)
Sample 1	17,601	97.1

5. If the original sample was diluted, multiply the sample Formaldehyde concentration by the sample dilution factor to determine the Formaldehyde concentration in the original sample.

EXAMPLE:

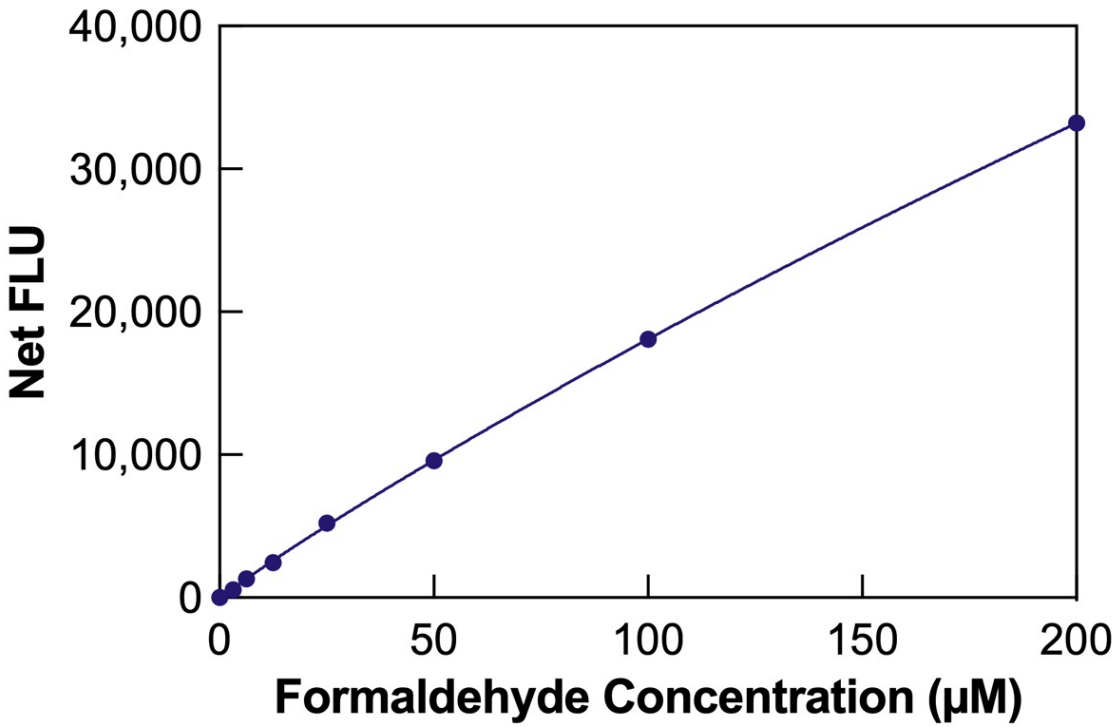
Sample	Formaldehyde Concentration (µM)	Sample Dilution Factor	Original Formaldehyde Concentration (µM)
Sample 1	97.1	4	388.4

TYPICAL DATA

⚠ **Always run your own standard curve. This data should NOT be used to interpret results.**

Sample	Mean FLU	Net FLU	Formaldehyde Concentration (µM)
Standard 1	33,981	33,195	200
Standard 2	18,844	18,058	100
Standard 3	10,347	9,561	50.0
Standard 4	6,001	5,215	25.0
Standard 5	3,228	2,442	12.5
Standard 6	2,104	1,318	6.25
Standard 7	1,334	548	3.13
Zero Standard	786	-	0.00
Sample 1	18,387	17,601	97.1
Sample 2	3,599	2,813	13.8

Typical Standard Curve



VALIDATION DATA

Sensitivity

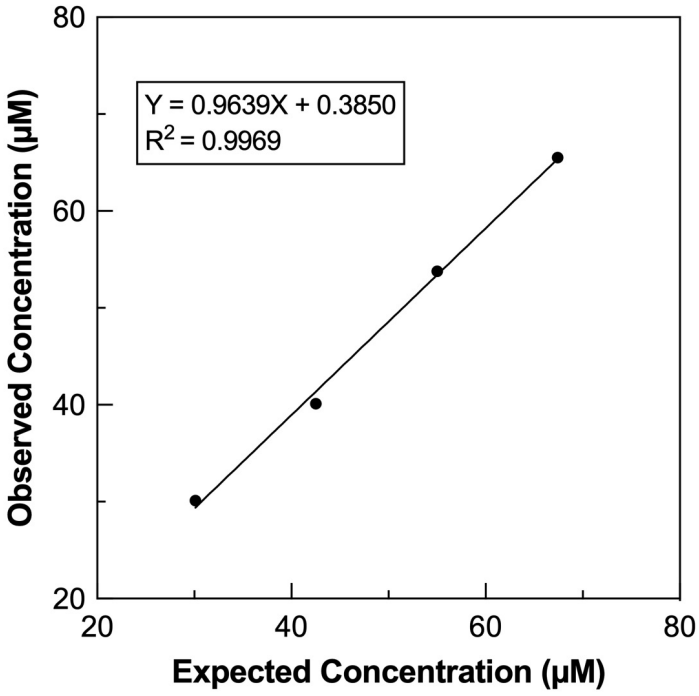
Sensitivity was calculated by comparing the fluorescence for twenty wells run for each of the Zero Standard and Standard #7. The detection limit was determined at two standard deviations from the Zero Standard along the standard curve.

Sensitivity was determined as 0.715 µM.

Linearity

Linearity was determined in human urine by mixing two samples with known Formaldehyde concentration in the ratios given below. One sample had a Formaldehyde concentration of 17.6 µM (Low Sample); a second sample had a Formaldehyde concentration of 79.9 µM (High Sample). The measured concentrations were compared to the expected values.

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



Intra Assay and Inter Assay Precision

For intra assay precision, four human urine samples were diluted 1:4 with deionized water and run in replicates of 20 in an assay. For 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. %CV represents the variation in concentration (not fluorescence) as determined using a standard curve.

	Intra Assay Precision		Inter Assay Precision	
Sample	Formaldehyde Concentration (µM)	% CV	Formaldehyde Concentration (µM)	% CV
1	9.70	7.3	10.5	6.7
2	38.3	4.2	36.9	4.5
3	76.0	3.4	71.1	3.8
4	162	3.7	148.9	4.3

SAMPLE VALUES

Eighteen clean catch urine samples were run in the assay. These samples were also run in the DetectX® Urinary Creatinine Detection kit, K002-H1/H5, and the Formaldehyde levels were normalized to the Creatinine concentration.

Sample Type	Average Adjusted Concentration (µM)	Adjusted Concentration Range (µM)	Normalized Concentration Range (µmol Formaldehyde per gram of Creatinine)
Urine	225	18 – 776	73 – 1,026

INTERFERENCE

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. The following list indicates the amount of each interferants known to inhibit the reaction of this assay.

Compound	Known Reaction Limit
Copper (II) Chloride	>1,000 µM
Copper (III) Chloride	>1 µM
Iron (III) Chloride	>1 µM
Iron (II) Sulfate	>1 µM
Sodium Bisulfite	>1 µM

CROSS REACTIVITY

The following inorganic compounds were diluted to 100 µM and tested in the assay.

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

TROUBLESHOOTING

Issue	Possible Cause & Solution
Reagent Shortage	• Check under the cap for additional reagent. Pulse spin reagent containers to collect contents prior to opening when possible. • When using a multichannel pipette, return unused reagent to container for later use.
Erratic Values	• Prerinse pipet tips with desired reagent prior to aspirating the required volume. • Deliver volume with care to prevent splashing into adjacent wells.
High Background	• Reagent contamination during assay setup. • Error in gain adjustment or auto-gain feature on spectrophotometer.
Low Signal	• Verify the plate reader wavelength is 510 nm with excitation at 450nm. • Room temperature incubation will yield approximately 75% of the fluorescent signal generated with 37ºC incubation

CITATIONS

1. US Consumer Product Safety Commission, Release #79-059. https://www.cpsc.gov/Newsroom/News-Releases/1980/Chemical-Industry-Test-Results-Show-Formaldehyde-Has-Caused-Cancer-In-Lab-Animals
2. International Agency for Research on Cancer, June 2004, www.iarc.fr/ENG/Press_Releases/archives/pr153a.html
3. Vaughan, Thomas L., et al. "Occupational exposure to formaldehyde and wood dust and nasopharyngeal carcinoma." Occupational and environmental medicine 57.6 (2000): 376-384.

RELATED PRODUCTS

Kits	Catalog No.
Urinary Creatinine Detection Kits	K002-H1/H5

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.

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CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us.

Arbor Assays

1143 Highland Dr, Ste A
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

www.ArborAssays.com

Email Addresses:

Info@ArborAssays.com

Orders@ArborAssays.com

Technical@ArborAssays.com

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 assay kits and reagents for wildlife conservation research.

PLATE LAYOUT