KAMIYA BIOMEDICAL COMPANY

Formaldehyde Fluorescent Detection Kit

For detection in human urine and tissue culture media

Cat. No. KT-723

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION

Formaldehyde Fluorescent Detection Kit

Cat. No. KT-708

Background

Formaldehyde (methanal), H2C=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 x 109 Kg are produced annually. 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. 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.

PRINCIPLE

The K-ASSAY® 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 calibrator is provided to generate a calibration curve for the assay and all samples should be read off the calibration curve. Calibrators or diluted samples are pipetted into a black microtiter plate. The fluorescent reaction is initiated with the 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.

COMPONENTS

- Black Half Area 96 Well Plates — 2 Plates
- Formaldehyde Calibrator — 0.5 mL
- Formaldehyde Reagent — 5 mL
- Plate Seals — 2 Seals

Storage

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

Other Materials Required

- Distilled or deionized water free of formaldehyde.
- Repeater pipet with disposable tips capable of dispensing 25 µL.
- An incubator capable of accurately maintaining 37℃.

- 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.
- 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 calibrators 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

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 calibration curve range should be diluted further with water to obtain readings within the calibration curve.

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

Use all diluted samples within 2 hours of preparation.

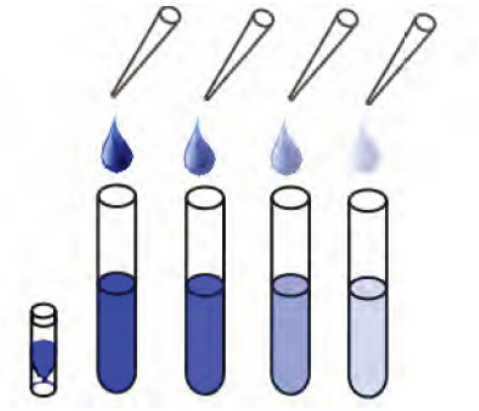
Reagent Preparation

Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all calibrators and samples be run in duplicate to allow the end user to accurately determine formaldehyde concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Calibrator Preparation

Label seven glass test tubes as #1 through #7. Pipet 450 µL of water into tube #1 and 250 µL into tubes #2-#7. Add 50 µL of the Formaldehyde stock solution to tube #1 and vortex completely. Take 250 µL of the formaldehyde solution in tube #1 and add it to tube #2 and vortex completely. Add 250 µL 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 µM. Water will be used as a sample blank.

Use all Calibrators within 2 hours of preparation.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 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 Conc (µM)	200	100	50	25	12.5	6.25	3.125

ASSAY PROTOCOL

1. A plate layout sheet has been included on the back page of the insert to aid proper sample and calibrator identification. Set plate parameters for a 96-well Corning Costar 3694 plate.
2. Pipet 50 µL of samples, water as the blank or calibrators into wells in the black plate.
3. Add 25 µL of the 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 calibrator and sample. Create a calibration curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean FLUs for the zero calibrator. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

Typical Data

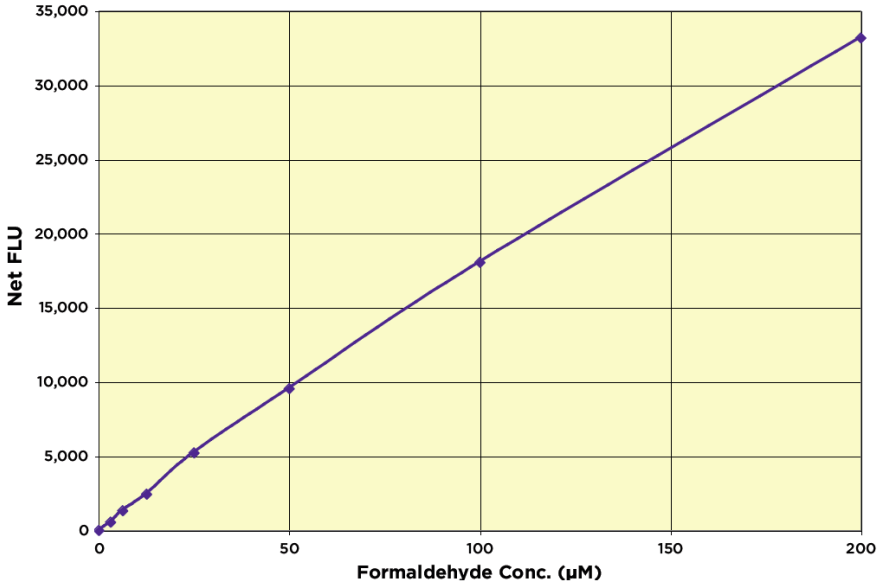
Always run your own calibration curve for calculation of results.

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

Do not use this data.

Typical Calibration Curve

Always run your own calibration curve for calculation of results.



Do not use this data.

Sensitivity

Sensitivity was calculated by comparing the FLU's for twenty wells run for each of the zero and calibrator #7. The detection limit was determined at two (2) standard deviations from the Zero along the calibration curve.
Sensitivity was determined as 0.715 µM

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

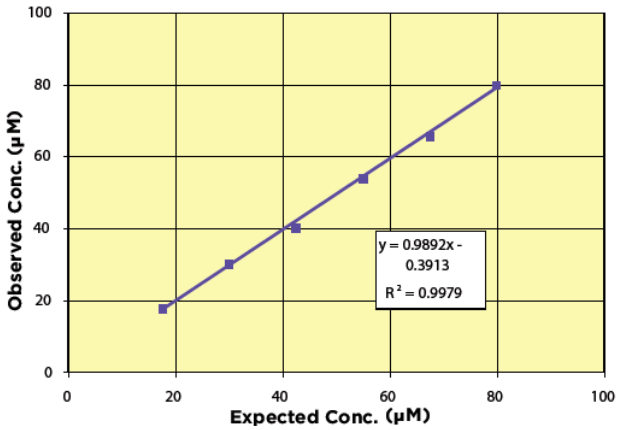
Four human urine samples were diluted 1:4 with deionized water and run in duplicates in 20 assays run over two days by two operators. The mean and precision of the calculated formaldehyde concentrations were:

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

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
100%	0%	17.6	--	--
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
0%	100%	79.9	--	--
			Mean Recovery	**97.4%**



Sample Values

Eighteen random clean catch urine samples were run in the assay. Formaldehyde concentrations in the neat urine ranged from 18 to 776 µM with an average of 225 µM. These samples were also run in the DetectX® Urinary Creatinine Detection kit, K002-H1/H5, and the formaldehyde levels normalized to creatinine concentration. Normalized values ranged from 73.0 to 1,026 µmoles 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 µM), diluted to 100µM 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, Na2SO3, 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 µM) and diluted to below 1 µM 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 µM
Copper(III) Chloride >1 µM
Iron(III) Chloride >1 µM
Iron(II) Sulfate >1 µM
Sodium Bisulfite >1 µM

H	G	F	E	D	C	B	A	
								1
								2
								3
								4
								5
								6
								7
								8
								9
								10
								11
								12

FOR RESEARCH USE ONLY

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