Formaldehyde Fluorescent Detection Kit

Catalog Number EIACH2O (192 tests)

Rev 1.0

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Formaldehyde Fluorescent Detection Kit is a fluorescent detection assay designed to measure the level of formaldehyde in tissue culture media and urine samples. The fluorescent reaction is initiated with the Formaldehyde Reagent which produces a fluorescent signal (450 nm excitation, 510 nm emission) when added to formaldehyde containing samples. The assay was characterized with human samples, but can be used with samples from other species.

Formaldehyde is a common by-product formed in the oxidative demethylation of proteins, nucleic acids, and biological small molecules. Materials containing formaldehyde can release formaldehyde gas or vapor into the air. It can also be released by burning wood, kerosene, natural gas, or cigarettes, from automobile emissions, and from natural processes. Exposure occurs primarily by inhaling formaldehyde vapor from the air or by absorbing liquids containing formaldehyde through the skin.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity
Formaldehyde Standard; 2,000 µM formaldehyde solution in a special stabilizing solution, **keep tightly sealed**	500 µL
Black 96-well Half Area Plate	2 plates
Formaldehyde Reagent; contains 0.09% sodium azide as a preservative	5 mL
Plate Sealer	2

Materials required but not supplied

- Distilled or deionized water
- Fluorescence microtiter plate reader with software capable of measurement at or near 510 nm, with excitation at 450 nm
- 37°C incubator
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
- **Important**: Formaldehyde is a toxic, volatile, reactive chemical. Use in a well-ventilated laboratory. Dispose of all excess standards and samples in a 10% aqueous solution of sodium bisulfite, or according to the appropriate institutional guidelines.

Sample preparation guidelines

If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

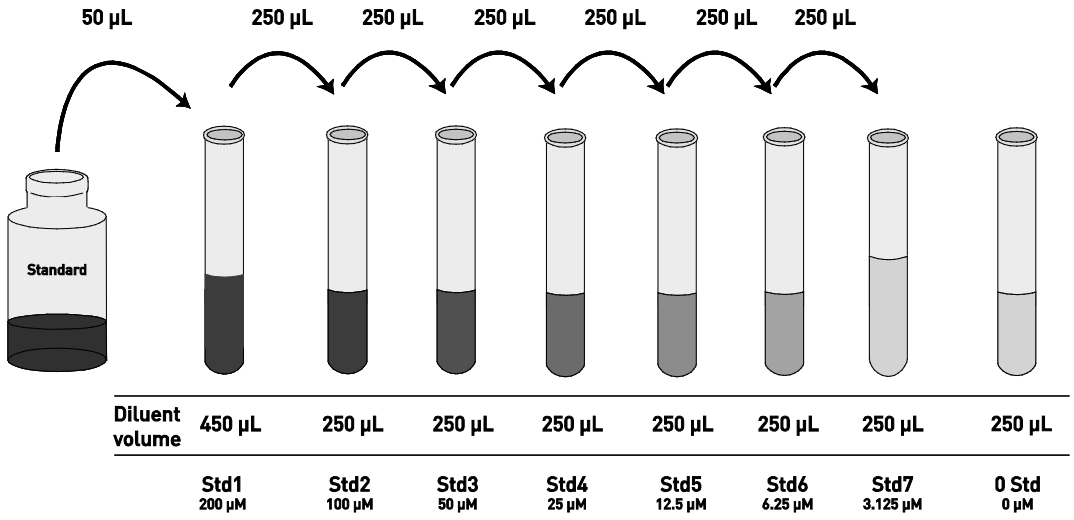
- Dilute **urine** samples 1:4 in distilled or deionized water.
- Dilute **tissue culture media** samples with the corresponding tissue culture medium.
- **Use all samples within 2 hours of dilution.**

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Important: For **tissue culture media** samples, dilute **standards with the appropriate tissue culture medium**.

1. Add 50 µL Formaldehyde Standard to one tube containing 450 µL distilled or deionized water and label as 200 µM formaldehyde.
2. Add 250 µL distilled or deionized water to each of 7 tubes labeled as follows: 100, 50, 25, 12.5, 6.25, 3.125, and 0 µM formaldehyde.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.



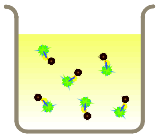
Assay procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 30 minutes.**

IMPORTANT! Perform a standard curve with each assay.

Add sample

Add 50 µL of standards or diluted samples (see page 2) to the appropriate wells.



Add fluorescent detection reagent

a. Add 25 µL Formaldehyde Reagent into each well.
b. Tap sides of the plate and mix. Cover the plate with a plate sealer.
c. Incubate for 30 minutes at 37°C.

Note: incubation at room temperature results in ~25% lower signal intensity.

Read the plate and generate the standard curve

1. Read the fluorescent emission at 510 nm, with excitation at 450 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background fluorescence may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in the appropriate diluent and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–200 µM formaldehyde.

Standard formaldehyde (µM)	Mean FLU
200	33,981
100	18,844
50	10,347
25	6,001
12.5	3,228
6.25	2,104
3.125	1,334
0	786

Intra-assay precision

Four human samples diluted 1:4 with deionized water were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3	Sample 4
Mean (µM)	9.70	38.3	76.0	16.2
%CV	7.3	4.2	3.4	3.7

CV = Coefficient of Variation

Inter-assay precision

Four human samples diluted 1:4 with deionized water were assayed 20 times in duplicate by two operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3	Sample 4
Mean (µM)	10.5	36.9	71.1	148.9
%CV	6.7	4.5	3.8	4.3

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Eighteen random clean catch samples were tested in the assay.

Sample	Range (µM)	Average (µM)
Urine	18–776 [1]	225
[1] When normalized with the Creatinine Urinary Detection Kit (Cat. No. EIACUN), the values ranged from 73–1,026 µmol of formaldehyde/mg creatinine.		

Interferents

A variety of solvents were tested as possible interfering substances in the assay by reacting with the formaldehyde present in the sample.

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 (µM)
Copper(II) Chloride	>1,000
Copper(III) Chloride	>1
Iron(III) Chloride	>1
Iron(II) Sulfate	>1
Sodium Bisulfite (Na_2SO_3)	>1

Linearity of dilution

Linearity was determined by assaying high and low concentration urine samples (high sample 79.9 µM formaldehyde; low sample 17.6 µM formaldehyde), mixed in the ratios shown in the following table.

Low Sample %	High Sample %	Expected Conc. (µM)	Observed Conc. (µM)	% Recovery
100	0	—	17.6	—
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
0	100	—	79.9	—
			Mean Recovery	**97.4%**

Sensitivity

The analytical sensitivity of the assay is 0.715 µM formaldehyde. This was determined by adding two standard deviations to the mean FLU obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Specificity

A variety of aldehydes, ketones, and inorganic compounds at a concentration of 100 µM were tested for cross-reactivity 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

Limited product warranty

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Catalog Number

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Temperature limitation


Use by


Manufacturer


Consult instructions for use


Caution, consult accompanying documents

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