ab133084 –

Formaldehyde Assay Kit

Instructions for Use

A convenient fluorescence-based method for detecting formaldehyde in urine.

This product is for research use only and is not intended for diagnostic use.
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1. Overview

ab133084 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.

Figure 1. Assay scheme
2. Background

Formaldehyde (CH₂O, 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. Formaldehyde is also generated during microsomal cytochrome P450-dependent oxidation of xenobiotics. It is also a product of oxidative deamination of methylamine catalyzed by semicarbazide-sensitive amine oxidase (SSAO). Increased SSAO-mediated deamination has been implicated in some pathophysiological conditions, such as diabetic complications.

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.

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. 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.

3. Components and Storage

This kit will perform as specified if stored at room temperature.

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde Detector</td>
<td>1 vial/150mg</td>
</tr>
<tr>
<td>Formaldehyde Ammonium Acetate</td>
<td>1 vial/5 ml</td>
</tr>
<tr>
<td>Formaldehyde DMSO</td>
<td>1 vial/2 ml</td>
</tr>
<tr>
<td>Formaldehyde Standard</td>
<td>1 vial/100 µl</td>
</tr>
<tr>
<td>Half Volume 96-Well Solid Plate (black)</td>
<td>1 plate</td>
</tr>
<tr>
<td>96-Well Cover Sheet</td>
<td>1 cover</td>
</tr>
</tbody>
</table>
Materials Needed But Not Supplied

- A fluorometer with the capacity to measure fluorescence using excitation wavelength between 365-375 nm and emission wavelength between 465-475 nm

- Adjustable pipettes and a repeat pipettor

- A source of pure water; glass distilled water or HPLC-grade water is acceptable
4. Pre-Assay Preparation

A. Reagent Preparation

**Formaldehyde Detector**

The vial contains 150 mg of acetoacetanilide. To prepare for use in the assay, weigh 42 mg into another vial, add 200 µl of Formaldehyde DMSO, and vortex until dissolved. Add 200 µl 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.

**Formaldehyde Ammonium Acetate**

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

**Formaldehyde DMSO**

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

The vial contains 100 µl 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.

B. Sample Preparation

Urine

Typically, Human urine has a formaldehyde concentration in the range of 40-1,400 µM.


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 diluted 1:2-1:5 with HPLC-grade water before assaying.
5. Assay Protocol

A. Plate Setup

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. A typical layout of samples to be measured in duplicate is given below in Figure 2.

![Plate Layout Diagram]

A-H – Standards
S1-S20 – Sample wells
B1-B20 – Background sample wells
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 µl 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.
B. **Standard preparation**

1. Dilute 10 µl of the Formaldehyde Standard with 9.99 ml of HPLC-grade water to yield a concentration of 3 mM.

2. Dilute 200 µl of the 3 mM Formaldehyde with 1.8 ml of HPLC-grade water to yield the stock solution of 300 µM. The 300 µM Standard will be used to prepare the standards.

3. Take eight clean glass test tubes and mark them A-H. Add the amount of Formaldehyde Standard (300 µM) and HPLC-grade water to each tube as described in Table 1. The diluted standards are stable for four hours at room temperature.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Formaldehyde 300 µM Standard (µl)</th>
<th>HPLC-grade water (µl)</th>
<th>Final Concentration (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>990</td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>980</td>
<td>6</td>
</tr>
<tr>
<td>D</td>
<td>60</td>
<td>940</td>
<td>18</td>
</tr>
<tr>
<td>E</td>
<td>100</td>
<td>900</td>
<td>30</td>
</tr>
<tr>
<td>F</td>
<td>200</td>
<td>800</td>
<td>60</td>
</tr>
<tr>
<td>G</td>
<td>400</td>
<td>600</td>
<td>120</td>
</tr>
<tr>
<td>H</td>
<td>500</td>
<td>500</td>
<td>150</td>
</tr>
</tbody>
</table>

Table 1. Formaldehyde standards
C. Performing the Assay

1. Standard Wells - add 50 µl of standard (tubes A-H) and 40 µl of Ammonium Acetate per well in the designated wells on the plate (see Figure 2).

2. Sample Wells - add 50 µl of sample and 40 µl 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 µl of HPLC-grade water, 50 µl of sample, and 40 µl of Ammonium Acetate to two wells.

4. Initiate the reactions by adding 10 µl 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.
6. Data Analysis

A. 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 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.

\[
\text{Formaldehyde (µM) = } \left( \frac{\text{CSF} - \text{(y-intercept)}}{\text{Slope}} \right) \times \text{Sample dilution}
\]
B. 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.
C. 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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde (500 µM)</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Propionaldehyde (500 µM)</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Acetone (1 mM)</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Methanol (3 mM)</td>
<td>&lt;0.001%</td>
</tr>
<tr>
<td>Magnesium Chloride (1 mM)</td>
<td>&lt;0.001%</td>
</tr>
<tr>
<td>Sodium Chloride (25 mM)</td>
<td>&lt;0.001%</td>
</tr>
<tr>
<td>Potassium Chloride (5 mM)</td>
<td>&lt;0.001%</td>
</tr>
<tr>
<td>Calcium Chloride (100 µM)</td>
<td>&lt;0.001%</td>
</tr>
<tr>
<td>β-hydroxybutyrate (500 µM)</td>
<td>&lt;0.001%</td>
</tr>
</tbody>
</table>
### 7. Troubleshooting

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