



# **Formaldehyde Microplate Assay Kit**

## **User Manual**

**Catalog # CAK1251**

(Version 1.3A)

Detection and Quantification of Formaldehyde Content in Urine,  
Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and  
Other biological fluids Samples.

**For research use only. Not for diagnostic or therapeutic procedures.**

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## I. INTRODUCTION

Formaldehyde is the simplest aldehyde. It is widely employed in industry for wide range of applications. Formaldehyde is also used as a disinfectant and is a commonly utilized tissue fixative and embalming agent. Formaldehyde is naturally present in all tissues and body fluids. Recently it has been shown that some cancer types exhibit elevated formaldehyde levels. Increased formaldehyde concentration in urine has been associated with prostate and bladder cancer. Thus, measuring formaldehyde in urine can be a very useful tool when studying cancer.

Formaldehyde Microplate Assay Kit provides a convenient tool for sensitive detection of Formaldehyde concentration in a variety of samples. The intensity of the product color, measured at 430 nm, is proportional to the Formaldehyde concentration in the sample.

## II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 2	4 °C
Assay Buffer II	30 ml x 2	4 °C
Substrate	Powder x 1	4 °C
Reaction Buffer	10 ml x 1	4 °C
Dye Reagent	0.1 ml x 1	4 °C
Standard (100 mmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

### Note:

**Substrate:** add 1 ml Reaction Buffer to dissolve before use.

**Dye Reagent:** add 0.9 ml Reaction Buffer, mix before use.

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 430 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

#### IV. SAMPLE PREPARATION

##### 1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Distilled water for  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10000g for 10 minutes, add 0.1 ml the supernatant into a new centrifuge tube and then add 0.45 ml Assay Buffer I, mix; centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube and then add 0.45 ml Assay Buffer II, mix.

##### 2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Distilled water, centrifuged at 10000g for 10 minutes, add 0.1 ml the supernatant into a new centrifuge tube and then add 0.45 ml Assay Buffer I, mix; centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube and then add 0.45 ml Assay Buffer II, mix.

##### 3. For serum, plasma, urine and other biological fluids samples

Add 0.1 ml sample into a centrifuge tube and then add 0.45 ml Assay Buffer I, mix; centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube and then add 0.45 ml Assay Buffer II, mix.

## V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	100µl	--	--
Standard	--	100µl	--
Distilled water	--	--	100µl
Reaction Buffer	80 µl	80 µl	80 µl
Substrate	10µl	10µl	10µl
Dye Reagent	10µl	10µl	10µl
Mix, cover the plate adhesive strip, incubate at 50°C for 15 minutes, record absorbance measured at 430 nm.			

### Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- 3) Reagents must be added step by step, can not be mixed and added together.

## VI. CALCULATION

1. According to the weight of sample

$$\begin{aligned} \text{Formaldehyde } (\mu\text{mol/g}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \\ &\quad / (W \times V_{\text{Sample}} / V_{\text{Assay}}) \times n \\ &= 1000 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

2. According to the quantity of cells or bacteria

$$\begin{aligned} \text{Formaldehyde } (\mu\text{mol}/10^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \\ &\quad \text{OD}_{\text{Blank}}) / (N \times V_{\text{Sample}} / V_{\text{Assay}}) \times n \\ &= 1000 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

3. According to the volume of sample

$$\begin{aligned} \text{Formaldehyde } (\mu\text{mol/ml}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \\ &\quad \text{OD}_{\text{Blank}}) / V_{\text{Sample}} \times n \\ &= 1000 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

$C_{\text{Standard}}$ : the concentration of standard, 100mmol/L = 100 $\mu$ mol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N  $\times 10^4$ ;

$V_{\text{Standard}}$ : the volume of standard, 0.1 ml;

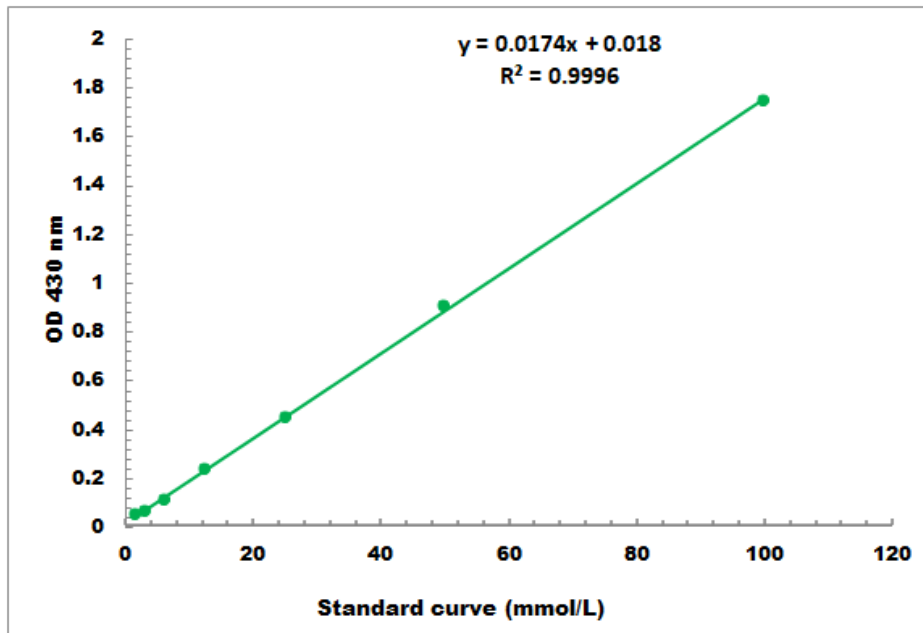
$V_{\text{Sample}}$ : the volume of sample, 0.1 ml;

$V_{\text{Assay}}$ : the volume of distilled water, 1 ml;

n: the dilution factor, 10.

## VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 1 mmol/L - 100 mmol/L

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.cohesionbio.com](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES