Formaldehyde Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6837

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 .

1. 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 andembalming 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 Colorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of f ormaldehyde 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.

2. 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 mlx 1	4 °C, keep in dark
Standard (10mol/L)	0.1 mlx 1	4 °C, keep in dark
Plate Adhesive Strips	3 strips	-
Technical Manual	1 Manual	-

3. 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. Hot air circulation oven

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dilute to 100 mmol/ Lstandard topsolution by adding 10 µl 10mol/ Lstandardstock solution into 99 0µl distilled water.Then perform 2 -fold serial dilutions of the top standardsolution using distilled water to make the standard curve.The concentration of standard curve could be 100/50/25/12.5/6.25/3.12/1.56mmol/ L.Prepare the top and standard curve solutionfor immediateuse.

Substrate: Briefly centrifuge prior to opening. Add 1 mL Reaction Buffer to dissolve before use. Store at 4°C for 3-6 months or -20°C for 1 year.

Dye Reagent: Briefly centrifuge prior to opening. Add 0.9 mL Reaction Buffer , mix before use. Keep in dark and store at 4°C for 2-3 weeks or -20°C for 3-6 months.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

5. 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 5×10^6 cell or bacteria, sonicate (with power 20 %, sonicate 3s, interval 10s ,repeat 30 times); centrifuged at 10000 g for 10minutes , add 0.1 mL the supernatant into a new centrifuge tube and then add 0. 45 mL Assay Buffer I, mix ; centrifuged at 10000 g for 10minutes ,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 10000 g for 10minutes , add 0.1 mL the supernatant into a new centrifuge tube and then add 0. 45 mL Assay Buffer I, mix; centrifuged at 10000 g for 10minutes ,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 10000 g for 10minutes ,take the supernatant into a new centrifuge tube and then add 0. 45 mL Assay Buffer II, mix.

6. Assay Procedure

Add following reagents in to 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 at50°C for 15minutes, recordabsorbance measured at 430 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

7. Calculation

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of

the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \ (mmol/L)$$

Calculate the initial concentration according to sample preparation procedure .

2. According to one point of the standard OD and concentration

2.1According to the quantity of cells or bacteria

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay})} \ (\mu mol/10^4)$$

2.2 According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay})} \ (\mu mol/g)$$

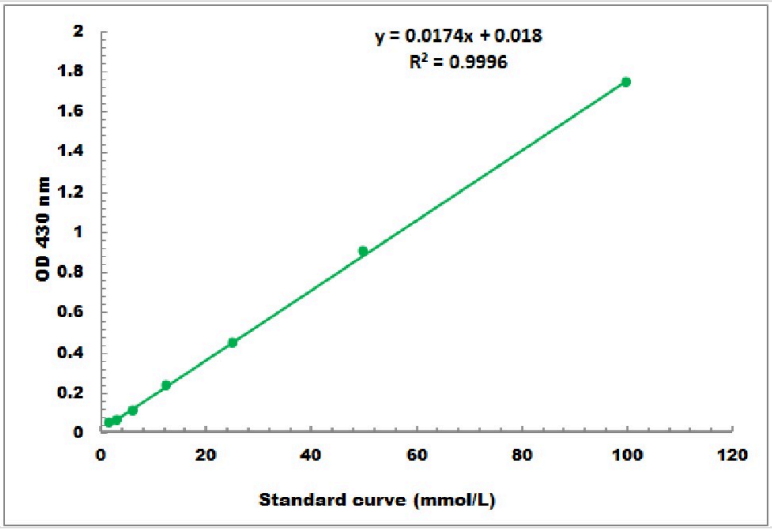
2.3According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V \times V_{Sample} / (V + V_{Assay})} \ (\mu mol/ml)$$

Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Standard}$	the standard concentration, mmol/L = μmol/ml
$V_{Standard}$	the volume of standard in assay procedure, μl
V_{Sample}	the volume of sample in assay procedure, μl
V_{Assay}	the volume of Assay Buffer I and Assay Buffer II , μl
W	the weight of sample, g
V	the volume of sample in sample preparation, ml
N	the quantity of cell or bacteria, 10^4

8. 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