Formaldehyde Assay Kit

Catalog No.: abx298964

Size: 100 Assays

Storage: Store all components at 4°C.

Application: For quantitative detection of Formaldehyde concentrations in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants and other biological fluids.

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

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.

Abbexa's Formaldehyde Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Formaldehyde concentrations. The dye reagent reacts with Formaldehyde to create an absorption maximum at 430 nm. The intensity of the color is proportional to the concentration of Formaldehyde, which can then be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer 1: 2 × 30 ml
3. Assay Buffer 2: 2 x 30 ml
4. Substrate: 1 vial
5. Reaction Buffer: 1 x 10 ml
6. Dye Reagent: 1 x 0.1 ml
7. Standard: 1 x 1 ml
8. Plate sealer: 3

Materials Required But Not Provided

1. Microplate reader (430 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Sonicator
7. Mortar
8. Incubator

Protocol

Preparation of Sample and Reagents

Reagents

- **Substrate Solution**
  Add 1 ml of Reaction Buffer into the Substrate vial and mix thoroughly to prepare the Substrate solution. Ensure that the Substrate has completely dissolved prior to use.

- **Dye Reagent Solution**
  Add 0.9 ml of Reaction Buffer into the Dye Reagent and mix thoroughly to prepare the Dye Reagent Solution.

Sample

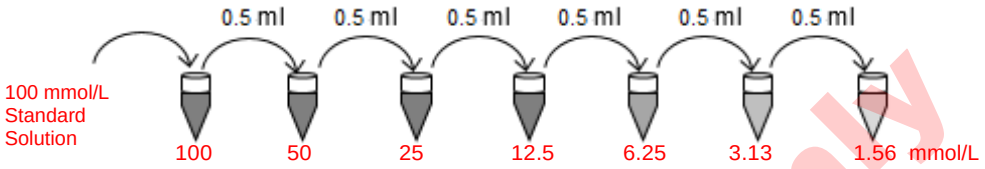
- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 1 ml of distilled water for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 10,000 × g for 10 minutes. Transfer 100 µl of the supernatant to a new tube, then add 450 µl Assay Buffer 1 and mix by gently inverting the tube. Centrifuge at 10,000 x g for 10 minutes. Put the supernatant into a new centrifuge tube and add 450 µl Assay Buffer 2. Mix by gently inverting the tube.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of distilled water. Centrifuge at 10,000 × g for 10 minutes. Transfer 100 µl of the supernatant to a new tube, then add 450 µl Assay Buffer 1 and mix by gently inverting the tube. Centrifuge at 10,000 x g for 10 minutes. Put the supernatant into a new centrifuge tube and add 450 µl Assay Buffer 2. Mix by gently inverting the tube.

- **Liquid samples**
  Transfer 100 µl sample to a microcentrifuge tube, then add 450 µl Assay Buffer 1 and mix by gently inverting the tube. Centrifuge at 10,000 x g for 10 minutes. Put the supernatant into a new centrifuge tube and add 450 µl Assay Buffer 2. Mix by gently inverting the tube.

Assay Procedure
If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 7 tubes with 100 mmol/L, 50 mmol/L, 25 mmol/L, 12.5 mmol/L, 6.25 mmol/L, 3.13 mmol/L and 1.56mmol/L. 1 ml of 25 mmol/L Standard Solution to the 1st tube and mix thoroughly. Aliquot 0.5 ml of distilled water into each of the remaining tubes. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 100 µl of sample to a sample wells.
4. Add 100 µl of prepared standards to standard wells.
5. Add 100 µl of distilled water to the blank wells.
6. Add 80 µl of Reaction buffer to all wells.
7. Add 10 µl of Substrate to all wells.
8. Add 10 µl of Dye Reagent to all wells.
9. Gently tap the plate to mix and cover the plate with a plate sealer.
10. Incubate the microplate at 50°C for 15 minutes.
11. Read and record absorbance at 430 nm.

B. Calculations

Formaldehyde concentration per g of sample:

$$\textbf{Formaldehyde}\ (\mu\text{mol/g}) = \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{n\,(V_{Sample} \times W)} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{1000}{W} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

Formaldehyde concentration per 10^4 cells or bacteria:

$$\textbf{Formaldehyde}\ (\mu\text{mol}/10^4\ \textbf{cells}) = \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{n\,(V_{Sample} \times N)} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{1000}{N} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

Formaldehyde concentration per ml serum or plasma:

$$\textbf{Formaldehyde}\ (\mu\text{mol/ml}) = \frac{C_{Standard} \times V_{Standard}}{n \times V_{Sample}} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = 1000 \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

where:

$C_{Standard}$	Concentration of highest standard (100 mmol/L= 100 µmol/ml)
W	Weight of the sample (in g)
N	Number of cells or bacteria (× 10^4)
V_{Assay}	Volume of Assay Buffer (1 ml)
V_{Sample}	Volume of sample (0.1 ml)
$V_{Standard}$	Volume of standard (0.1 ml)
n	The dilution factor (10)