Formaldehyde Assay Kit (Fluorometric)

Formaldehyde Assay Kit (Fluorometric) is a detection kit for the quantification of Formaldehyde in biological, food, beverage and environmental samples.

Catalog number: ARG82153

Package: 100 tests

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INTRODUCTION

Formaldehyde (systematic name methanal) is a naturally occurring organic compound with the formula CH_2O (H–CHO). The pure compound is a pungent-smelling colorless gas that polymerises spontaneously into paraformaldehyde (refer to section Forms below), hence it is stored as an aqueous solution (formalin). It is the simplest of the aldehydes (R–CHO). The common name of this substance comes from its similarity and relation to formic acid.

Formaldehyde is an important precursor to many other materials and chemical compounds. In 1996, the installed capacity for the production of formaldehyde was estimated at 8.7 million tons per year. It is mainly used in the production of industrial resins, E.g., for particle board and coatings.

In view of its widespread use, toxicity, and volatility, formaldehyde poses a significant danger to human health. In 2011, the US National Toxicology Program described formaldehyde as "known to be a human carcinogen". [Provide by Wikipedia: Formaldehyde]

PRINCIPLE OF THE ASSAY

This Formaldehyde Assay Kit (Fluorometric) is a simple fluorometric assay that measures the amount of formaldehyde present in biological samples. In the assay, formaldehyde is derivatized with acetoacetanilide in the presence of ammonia. The resulting fluorescent product is then quantified fluorimetrically (λexc/em = 370/470 nm).

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped at room temperature. Store all components at 4°C. Shelf life of 18 months after receipt.

Component	Quantity	Storage information
Reagent A	5 mL	4°C
Reagent B	3 mL	4°C
10 % TCA	5 mL	4°C
Neutralizer	2 x 1.5 mL	4°C
Standard (10 mM Formaldehyde)	100 µL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microplate reader capable of reading excitation at 370 nm and emission at 470 nm
- Centrifuge
- Deionized or Distilled water
- Black flat-bottom 96 well microplate
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

Urine: Urine samples should be diluted 2-5 fold with distilled water. If urine samples contain visible particulates, then the samples should be centrifuged for 5 minutes at 14000 rpm.

Note:

- High protein sample (cell lysate, serum, etc.) need to be deproteinated and neutralized prior to assaying. To deproteinate, add 50 µL of 10% TCA per 100 µL sample. Vortex and centrifuge for 5 minutes at 14000 rpm. Transfer 100 µL of clear supernatant to a clean tube and neutralize with 25 µL of Neutralizer.
- Measured ΔRFU's for deproteinated samples need to be multiplied by 1.875 to compensate for the resulting dilution of the sample.
- Samples not measured the same day should be stored frozen, preferably at-80°C.

REAGENT PREPARATION

- **Working Reagent:** for each reaction, mix 33 µL of Reagent A and 22 µL of Reagent B. For the Sample Blanks, make the following Working Reagent: 33 µL of Reagent A + 22 µL of distilled water.
- **Standards:** Mix 5 µL of the provided 10 mM Formaldehyde with 495 µL of distilled water to make a 100 µM Premix. Dilute standard as follows.

Standard tube	Formaldehyde (µM)	Distilled water (µL)	Standard Premix (µL)
S1	100	0	100
S2	60	40	60
S3	30	70	30
S4	0	100	0

ASSAY PROCEDURE

Prior to the assay, equilibrate all components to room temperature. Briefly centrifuge tubes before opening.

1. Use a fluorescent, flat-bottom, black 96-well microplate for assay.
2. Add **50 µL** of **Standards** in to separate wells.
3. Add **50 µL** of each **prepared sample** to **two separate wells**. One well will be used as a Sample Blank.
4. Add **50 µL** of the **appropriate Working Reagent** to each well. Tap plate to mix. Incubate for **30 minutes** at **room temperature** in the dark.
5. Read the plate immediately with a fluorescence microplate reader using **excitation 370 nm filter** and **emission 470 nm filter.**

CALCULATION OF RESULTS

1. Plot the RFU measured at 30 minutes for each standard against the standard concentrations. Determine the slope using linear regression fitting. The Formaldehyde concentration of a Sample is calculated as follow:

   Formaldehyde (µM) = [(RFU_{SAMPLE} – RFU_{BLANK} – RFU_{WATER}) / Slope] x n

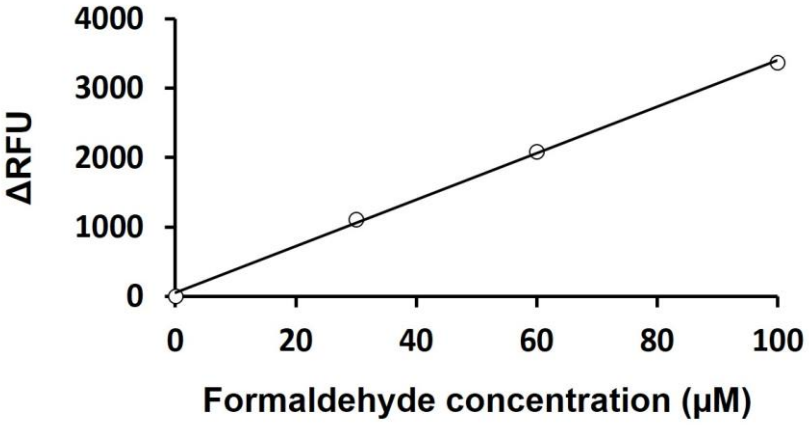
   Note:

   - RFU_{SAMPLE}, RFU_{BLANK} and RFU_{WATER}: the fluorescence values of sample, sample blank and S4.
   - Slope is the slope of the standard curve in μM^{-1} and n is the dilution factor (n = 1.875 for deproteinated samples).
   - If the Sample Formaldehyde concentration is higher than the 100 µM prior to applying the dilution factor, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

2. Conversion factor: 1 µM formaldehyde is equivalent to 30 ppb.

EXAMPLE OF RESULT

The following figures demonstrate typical results with the Formaldehyde Assay Kit (Fluorometric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

1.5 µM (45 ppb)