# Technical Manual

## FluoroDazzle Formaldehyde Assay Kit

## Catalogue Code: BA0022

## Pack Size: 100 assays

## Research Use Only

## DESCRIPTION

*FORMALDEHYDE* (methanal) 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.

The Assay Genie FluoroDazzle Formaldehyde Assay Kit provides a convenient fluorimetric means to measure formaldehyde in biological samples. In the assay, formaldehyde is derivatized with acetoacetanilide in the presence of ammonia. The resulting fluorescent product is then quantified fluorimetrically ($I_{exc/em}$ = 370/470nm). The assay is simple, sensitive, stable and high-throughput adaptable. The assay can detect as low as 1.5 µM formaldehyde in biological samples.

## KEY FEATURES

**Safe.** Non-radioactive assay.

**Sensitive and accurate.** As low as 1.5 µM (45 ppb) formaldehyde can be quantified.

**Homogeneous and convenient**. "Mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.

**Robust and amenable to HTS:** Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

## APPLICATIONS

**Formaldehyde determination** in urine and other biological samples.

## KIT CONTENTS

| | | | | | |
|---|---|---|---|---|---|
| **Reagent A:** | 5 mL | **Reagent B:** | 3 mL | **Standard:** | 100 µL |
| **10% TCA:** | 5 mL | **Neutralizer:** | 2 x 1.5 mL | | |

**Storage conditions:** The kit is shipped at room temperature. Store all reagents at 4°C. Shelf life of 18 months after receipt.

**Precautions:** 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.

## ASSAY PROCEDURE

Use black flat-bottom plates. Prior to assay, bring all reagents to room temperature.

1. Standards. Mix 5 µL the provided 10 mM Formaldehyde with 495 µL $dH_2O$ to make a 100 µM Premix. Dilute standard as follows.

| No | Premix + $H_2O$ | Formaldehyde (µM) |
|---|---|---|
| 1 | 100 µL + 0 µL | 100 |
| 2 | 60 µL + 40 µL | 60 |
| 3 | 30 µL + 70 µL | 30 |
| 4 | 0 µL + 100 µL | 0 |

Transfer 50 µL standards into separate wells of the plate.

2. Sample Preparation. Urine samples should be diluted 2-5 fold with $dH_2O$. If urine samples contain visible particulates, then the samples should be cleared by either filtration or centrifugation (14000 rpm, 5 min).

Samples high in protein (cell lysate, serum, etc.) need to be deproteinated and neutralized prior to assaying. To deproteinate, add 50 µL 10% TCA per 100 µL sample. Vortex and centrifuge for 5 min at 14000 rpm. Transfer 100 µL of clear supernatant to a clean tube and neutralize with 25 µL Neutralizer. *Note: 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.

3. Add 50 µL of each prepared sample to two separate wells of the plate (one well will be used as a Sample Blank).
4. Prepare Working Reagent for each standard and sample well by mixing 33 µL Reagent A and 22 µL Reagent B. For the Sample Blanks, make the following Working Reagent: 33 µL Reagent A + 22 µL $dH_2O$. Add 50 µL of the appropriate Working Reagent to each well. Tap plate to mix. Incubate at room temperature for 30 min protected from light.
5. Read fluorescence intensity at $\lambda_{exc}$ = 370 nm and $\lambda_{em}$ = 470 nm.

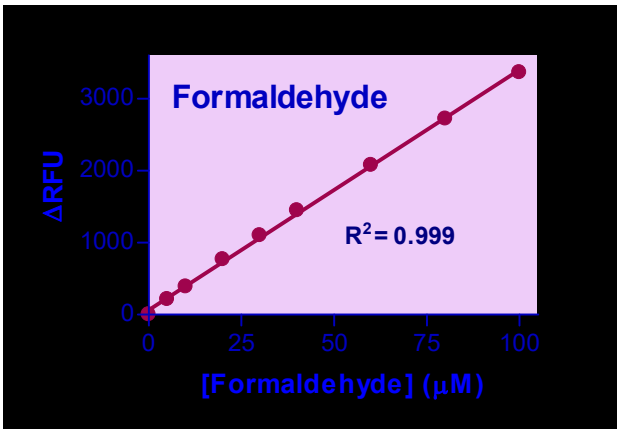
**CALCULATION**

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

$$\textbf{[Formaldehyde]} = \frac{\textbf{RFU}_{\textbf{SAMPLE}} - \textbf{RFU}_{\textbf{BLANK}} - \textbf{RFU}_{\textbf{WATER}}}{\textbf{Slope}} \times \boldsymbol{n} \quad (\mu\textbf{M})$$

where $\textbf{RFU}_{\textbf{SAMPLE}}$, $\textbf{RFU}_{\textbf{BLANK}}$ and $\textbf{RFU}_{\textbf{WATER}}$ are the measured fluorescence values of the sample, sample blank and water *(std #4)* respectively. **Slope** is the slope of the standard curve in $\mu M^{-1}$ and ***n*** is the dilution factor (***n*** = 1.875 for deproteinated samples). Note: 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.

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




Formaldehyde Standard Curve in Water

**MATERIALS REQUIRED, BUT NOT PROVIDED**

Pipetting devices, centrifuge tubes, black flat bottom 96-well plates (e.g. Corning Costar) and plate reader.

**LITERATURE**

1. Li, Q. et al. (2007). Development of Novel Reagent for Hantzsch Reaction for the Determination of Formaldehyde by Spectrophotometry and Fluorometry. Anal. Sci. 23: 413-7.
2. Li, Q. et al. (2007). Flow-Injection Spectrofluorometric Determination of Trace Amounts of Formaldehyde in Water after Derivatization with Acetoacetanilide. Talanta 72:1675-80.

3. Fan, Q. and Dasgupta, PK (1994). Continuous Automated Determination of Atmospheric Formaldehyde at the Parts Per Trillion Level. Anl. Chem. 66: 551-6.

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