3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Formaldehyde Assay Kit

Catalog Number **MAK131**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Formaldehyde is widely employed in industry for a wide range of applications. It acts as a disinfectant and biocide, and is utilized as a tissue fixative and embalming agent. Formaldehyde is a key metabolite produced during normal metabolism and contributes to methylation reactions and protein and nucleic acid synthesis. Increased production of formaldehyde has been observed in many cancer cell lines and increased formaldehyde concentration in urine has been associated with bladder and prostrate cancer.

The Formaldehyde Assay kit provides a simple and direct procedure for measuring formaldehyde levels in urine and other biological samples. In this assay, formaldehyde is derivatized with acetoacetanilide in the presence of ammonia resulting in a fluorescent (λ_{ex} = 370/λ_{em} = 470 nm) product, proportional to the formaldehyde present.

Components

The kit is sufficient for 100 assays in 96 well plates.

Reagent A Catalog Number MAK131A	5 mL
Reagent B Catalog Number MAK131B	3 mL
10% TCA Catalog Number MAK131C	5 mL
Neutralizer Catalog Number MAK131D	2 × 1.5 mL
Standard Catalog Number MAK131E	100 µL

Reagents and Equipment Required but Not Provided.

- Fluorometric multiwell plate reader
- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

This kit is shipped at ambient conditions and storage at 2–8 °C is recommended.

Procedure

Just prior to assay, bring all reagents to room temperature.

Sample Preparation

Urine samples should be diluted 2 to 5-fold with water. Urine samples containing visible particulates should be cleared by either filtration or centrifugation (5 minutes at 14,000 rpm).

Samples high in protein (cell lysate, serum, etc.) need to be deproteinated and neutralized prior to the assay. To deproteinate, add 50 µL of 10% TCA per 100 µL of sample. Vortex and centrifuge for 5 minutes at 14,000 rpm. Transfer 100 µL of clear supernatant to a clean tube and neutralize with 25 µL of Neutralizer.

Best results are obtained with freshly prepared samples. If this is not feasible, samples not measured the same day as preparation should be stored frozen at –80 °C.

Formaldehyde Standards for Fluorometric Detection

Mix 5 µL of the 10 mM Formaldehyde Standard with 495 µL of water to create a 100 µM working standard. Dilute the 100 µM working standard as indicated in Table 1.

Table 1.
Fluorometric Standards

Number	Standard	Water	Formaldehyde Concentration
1	100 µL	0 µL	100 µM
2	60 µL	40 µL	60 µM
3	30 µL	70 µL	30 µM
4	0 µL	100 µL	0 µM

Assay Reaction

1. Transfer 50 µL of each standard into separate wells of a 96 well plate.

2. Transfer 50 µL of each prepared sample to two separate wells of the plate (one well will be used as a sample blank).

3. Prepare Reaction Mixes according to the Scheme in Table 2. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 2.
Reaction Mixes

Reagent	Samples and Standards	Sample Blank
Reagent A	33 µL	33 µL
Reagent B	22 µL	–
Water	–	22 µL

4. Transfer 50 µL of the appropriate Reaction Mix to each of the sample and standard wells and tap plate briefly to mix.

5. Incubate the samples at room temperature for 30 minutes protected from light.

6. Measure fluorescence intensity (FLU) at λ_{ex} = 370/λ_{em} = 470 nm.

Calculations

Use the values obtained from the appropriate standards to plot a standard curve and determine the slope using linear regression fitting. The amount of formaldehyde present in the samples may be determined from the standard curve using the equations below.
Note: A new standard curve must be set up each time the assay is run.

Formaldehyde Concentration (µM)

$$= \frac{(FLU_{sample} - FLU_{blank}) - FLU_{water}}{\text{slope}} \times n$$

FLU_{water} = value for 0 standard
n = dilution factor (1.875 if samples were deproteinated according to the procedure within this protocol)

If the formaldehyde concentration of the sample without consideration of the dilution factor is >100 µM, dilute sample further in water and repeat the assay. Multiply the results by the dilution factor.

1 µM formaldehyde is equal to 30 ppb.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms.
Lower/higher readings in samples and standards	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

LS,MAM 12/13-1