Version 1 Last updated 12 May 2020

# ab272524 Formaldehyde Assay Kit

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Formaldehyde Assay Kit datasheet:
www.abcam.com/ab272524
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For quantitative determination of Formaldehyde in biological, food and environmental samples.

This product is for research use only and is not intended for diagnostic use.

# Table of Contents

# 1. Overview

Formaldehyde Assay Kit (ab272524) provides a convenient fluorometric 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 fluorometrically (λexc/λem = 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.

**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.

## 2. Protocol Summary

Prepare all reagents and samples as instructed.

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Add standards, blanks and samples to appropriate wells.

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Add appropriate Working Reagent (WR) to samples, blanks and standards.

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Incubate for 30 minutes at RT protected from light.

⇩

Read fluorescence at λex/λem = 370/470 nm.

## 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

## 4. Storage and Stability

**Store kit at 4°C immediately upon receipt. Kit has a storage time of 18 months from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| Reagent A | 5 mL | +4°C |
| Reagent B | 3 mL | +4°C |
| TCA 10% | 5 mL | +4°C |
| Neutralizer | 2 ×1.5 mL | +4°C |
| Standard | 100 µL | +4°C |

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Pipetting devices
- Tubes
- Centrifuge
- Heat block or water bath
- 96-well black plate with flat bottom
- Standard microplate reader - capable of reading fluorescence at λex/λem = 370/470 nm.

## 8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

## 9. Reagent Preparation

– Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 100 assays.

All reagents are supplied ready to use.

## 10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.

**10.1** Mix 5 µL the provided 10 mM Formaldehyde with 495 µL dH2O to make a 100 µM Premix. Dilute standard as follows:

| Standard # | Premix (µL) | $H_2O$ (µL) | Formaldehyde (µM) |
|---|---|---|---|
| 1 | 100 | 0 | 100 |
| 2 | 60 | 40 | 60 |
| 3 | 30 | 70 | 30 |
| 4 | 0 | 100 | 0 |

**10.2** Transfer 50 µL standards into separate wells of the plate

## 11. Sample Preparation

Suitable assay samples include: serum, plasma, whole blood, urine and saliva.

### Sample treatment

Urine samples should be diluted 2-5 fold with dH2O. If urine samples contain visible particulates, then the samples should be cleared by either filtration or centrifugation (14000 rpm, 5 min).

**Δ Note:** Serum, plasma, whole blood, cell culture media containing FBS, tissue or cell lysates require deproteination prior to assay (see below).

**Δ Note:** Urine and saliva do not require deproteination prior to assay.

### Deproteination:

Add 50 µL 10% TCA per 100 µL sample.
Vortex and centrifuge for 5 minutes at 14,000 rpm.
Transfer 100 µL of the clear supernatant to a clean tube and neutralize with 25 µL Neutralizer.

**Δ Note:** Note: Measured ΔRFU's for deproteinated samples need to be multiplied by 1.875 to compensate for the resulting dilution of the sample.

**Δ Note:** Samples not measured the same day should be stored frozen, preferably at -80°C.

## 12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

**12.1** Immediately prior to starting the reaction, prepare enough Working Reagent (WR) for all samples and standards by mixing per reaction tube: 33 µL Reagent A, 22 µL Reagent B.

| Component | Working Reagent (µL/reaction) For Samples and Standards |
|---|---|
| Reagent A | 33 |
| Reagent B | 22 |

**12.2** For the Sample Blanks, make the following Working Reagent: 33 µL Reagent A + 22 µL $dH_2O$.

| Component | Working Reagent (µL/reaction) For sample Blanks |
|---|---|
| Reagent A | 33 |
| $H_2O$ | 22 |

**12.3** Add 50µL of each prepared sample to two separate wells of the plate (one well will be used as a Sample Blank).

**12.4** Add 50µL of the appropriate Working Reagent to each well. Tap plate to mix.

**12.5** Incubate at room temperature for 30 min protected from light.

**12.6** Read fluorescence intensity at $\lambda_{exc}$ = 370 nm and $\lambda_{em}$ = 470 nm.

## 13. Calculations

**13.1** Plot the RFU measured at 30 min for each standard against the standard concentrations.

**13.2** Determine the slope using linear regression fitting.

**13.3** The Formaldehyde concentration of a Sample is calculated as:

$$[\text{Formaldehyde}] = \frac{(RFU_{Sample} - RFU_{Blank} - RFU_{water})}{\text{Slope}} \times n \quad (\mu M)$$

$RFU_{Sample}$ = value of the Sample
$RFU_{Blank}$ = value of the Sample Blank
$RFU_{water}$ = value of water
Slope = the standard curve slope in $\mu M^{-1}$
n = is the dilution factor (n = 1.875 for deproteinated samples)

**Δ Note:** f 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.

## 14. Typical Data

**Typical standard curve** – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.

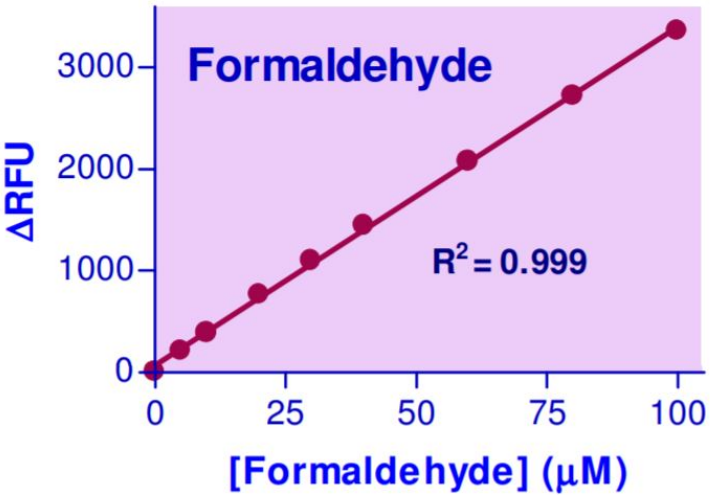


**Figure 1**. Example of Formaldehyde Assay Kit standard curve.

# 15. Notes

## Technical Support



### For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)