ab196997
Formaldehyde Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Formaldehyde in animal tissue, cells and biological fluids.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

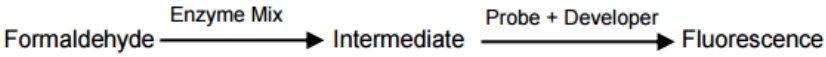
Version 3 Last Updated 30 October 2023

Table of Contents

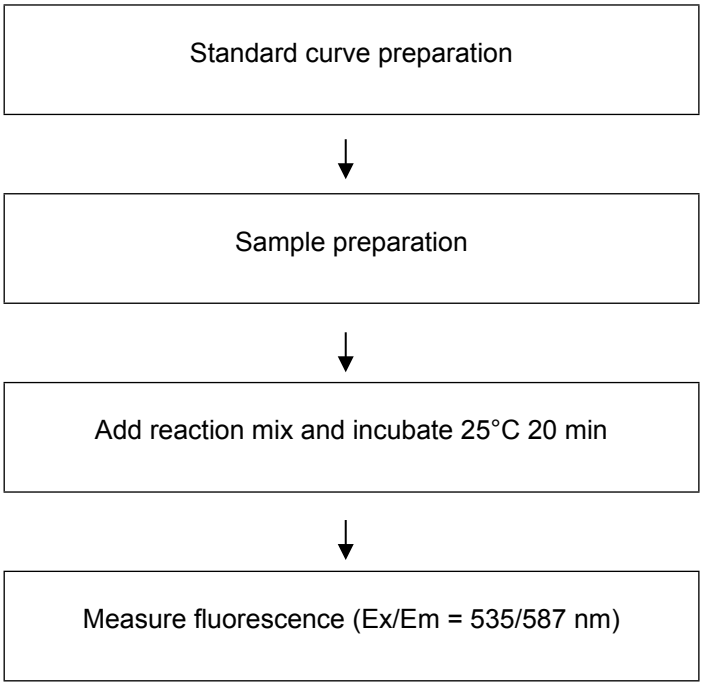
1. BACKGROUND

Formaldehyde Assay Kit (fluorometric) (ab196997) provides a simple, sensitive and high-throughput adaptable assay that detects biologically relevant concentrations of formaldehyde in various fluids and tissues. The assay is based on the oxidation of formaldehyde, producing a stable fluorescent signal, which is directly proportional to the amount of formaldehyde in samples. The kit can detect less than 2 µM of formaldehyde in a variety of samples.

Formaldehyde (HCHO) is the simplest aldehyde and one of the most often used organic compounds in industrial processes due to its high reactivity with a variety of chemicals. Endogenous formaldehyde can be produced in organisms via metabolic processes while exogenous formaldehyde can be absorbed after oral, dermal or inhalation exposure. Formaldehyde is highly toxic due to its capacity to covalently bind to macromolecules, such as DNA, and is well known for its neurotoxicological effects. Exposure to relatively high levels of formaldehyde is believed to cause leukemia, nose and nasopharyngeal cancer, etc.; however the epidemiological evidence is unclear. It can also affect memory and learning capacity.



2. ASSAY SUMMARY



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. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year 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 section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
HCHO Assay Buffer/Formaldehyde Assay Buffer	25 mL	-20°C	-20°C
PicoProbe I/PicoProbe (in DMSO)	400 µL	-20°C	-20°C
Formaldehyde Enzyme Mix/Formaldehyde Enzyme Mix (lyophilized)	1 vial	-20°C	-20°C
Developer Mix II/Formaldehyde Developer (lyophilized)	1 vial	-20°C	-20°C
Formaldehyde Standard/Formaldehyde Standard (100 mM)	100 µL	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter for Ex/Em = 535/587 nm
- 96 well plate: black plates with clear bottom for fluorometric assay
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)

For deproteinization step, additional reagents are required:

- Perchloric acid (PCA) 4M, ice cold
- Potassium Hydroxide (KOH) 2M
- 10 kD Spin Columns (ab93349) – for fluid samples, if not performing PCA precipitation

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- 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.

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.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **HCHO Assay Buffer/Formaldehyde Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C. Use within 2 months.

9.2 **PicoProbe I/PicoProbe – in DMSO:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.** Aliquot probe so that you have enough volume to perform the desired number of tests. Store at -20°C protected from light and moisture. Once the probe is thawed, use with two months. Keep on ice while in use.

9.3 **Formaldehyde Enzyme Mix:**

Reconstitute in 220 µL HCHO Assay Buffer/Formaldehyde Assay Buffer. Pipette gently to dissolve. Aliquot enzyme so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.4 **Developer Mix II/Formaldehyde Developer:**

Reconstitute in 220 µL HCHO Assay Buffer/formaldehyde Assay Buffer. Pipette gently to dissolve. Aliquot Developer Mix II/developer so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.5 **Formaldehyde Standard:**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot standard so that you have enough volume to perform the desired number of tests. Store at -20°C. Keep on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.

10.1 Prepare a 1 mM HCHO (formaldehyde) standard by diluting 10 µL of the provided 100 mM Formaldehyde Standard/standard with 990 µL of ddH_2O.

10.2 Prepare 50 µM standard by diluting 50 µL of 1 mM Formaldehyde Standard/HCHO standard with 950 µL of ddH_2O.

10.3 Using 50 µM Formaldehyde Standard/HCHO standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	HCHO Assay Buffer/Assay Buffer (µL)	Final volume standard in well (µL)	End Conc. HCHO in well
1	0	150	50	0 pmol/well
2	6	144	50	100 pmol/well
3	12	138	50	200 pmol/well
4	18	132	50	300 pmol/well
5	24	126	50	400 pmol/well
6	30	120	50	500 pmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step as well as the deproteinization step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of ice cold HCHO Assay Buffer/formaldehyde Assay Buffer.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Centrifuge sample for 2 – 5 minutes at 4°C at 10,000 x *g* using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.1.7 Keep on ice.

11.1.8 Perform deproteinization step as described in section 11.4.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of ice cold HCHO Assay Buffer.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.5 Centrifuge samples for 2 – 5 minutes at 4°C at 10,000 x *g* using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

11.2.7 Keep on ice.

11.2.8 Perform deproteinization step as described in section 11.4.

11.3 **Plasma, Serum and Urine and other biological fluids:**

Plasma, serum and urine samples generally contain high amount of proteins, so they should be deproteinized as described in section 11.4.

Alternatively, you can use 10kD Spin column (ab93349) to deproteinize biological fluids.

11.3.1 Centrifuge biological fluids (i.e. saliva, serum, plasma, urine) at 10,000 X g for 5 min. at 4°C.

11.3.2 Collect supernatant which can be tested directly by adding sample to the microplate wells.

11.3.3 Bring volumes up to 50 µL with HCHO Assay Buffer/Assay Buffer.

For urine samples, dilute urine 10-20X in HCHO Assay Buffer/Formaldehyde Assay Buffer. Add 5-50 µL of the dilute urine sample to reaction well.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (1/2 – 1/5 – 1/10).

11.4 **Deproteinization step:**

Prepare samples as specified in protocol. You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.

11.4.1 Add ice cold PCA 4 M to a final concentration of 1 M in the homogenate solution and vortex briefly to mix well. ***NOTE****: high protein concentration samples might need more PCA.*

11.4.2 Incubate on ice for 5 minutes.

11.4.3 Centrifuge samples at 13,000 x g for 2 minutes at 4°C in a cold centrifuge and transfer supernatant to a fresh tube. Measure volume of supernatant.

11.4.4 Precipitate excess PCA by adding an equal volume of ice-cold 2 M KOH to supernatant obtained in previous step and vortex briefly. This will neutralize the sample and precipitate excess PCA. After neutralization, it is very important that pH equals 6.5 – 8 (use pH paper to test 1 µL of sample). Any left over PCA will interfere with the assay.

11.4.5 Centrifuge at 13,000 x g for 15 minutes at 4°C and collect supernatant.

Samples are now deproteinized, neutralized and PCA has been removed. The samples are now ready to use in the assay.

Sample Recovery

The deproteinized samples will be diluted from the original concentration.

To calculate the dilution factor of your final sample, simply apply the following formula:

$$\%\ original\ concentration = \frac{initial\ sample\ volume}{(initial\ sample\ volume + volume\ PCA\ adde}$$

NOTE: *Formaldehyde concentrations can be widely variable. We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

Endogenous compounds in the sample may interfere with the reaction. To ensure accurate determination of formaldehyde in the test samples with low formaldehyde, we recommend spiking samples with a known amount of formaldehyde Standard (300 pmol).

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with HCHO Assay Buffer/Assay Buffer).
- Background control sample wells= 1 – 50 µL samples (adjust volume to 50 µL/well with HCHO Assay Buffer/Assay Buffer). ***NOTE:*** *for samples with compounds that are likely to generate high background.*

12.2 **Formaldehyde Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
HCHO Assay Buffer /Formaldehyde Assay Buffer	**43**	**45**
Formaldehyde Enzyme Mix	**2**	**0**
Developer Mix II/Formaldehyde Developer	**2**	**2**
PicoProbe I/PicoProbe	**3**	**3**

Mix enough reagents for the number of assays (samples and standards) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards +1).

12.3 Add 50 µL of Formaldehyde Reaction Mix into each standard and sample well.

12.4 Add 50 µL of background Mix into each sample background well.

12.5 Mix and incubate at 25°C for 20 minutes protected from light.

12.6 Measure fluorescence on a microplate reader at Ex/Em = 535/587 nm.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of formaldehyde (HCHO).

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Extrapolate sample readings from the standard curve plotted using the following equation:

$$B = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

13.7 Apply the corrected RFU to the HCHO standard curve to get B pmol of HCHO in the sample well.

13.8 Concentration of formaldehyde in the test samples is calculated as:

$$HCHO(pmol/\mu L or\ \mu M) = \left(\frac{B}{V}\right) * D$$

Where:

B = Amount of formaldehyde in the sample well (pmol).

V = Sample volume added into the reaction well (µL).

D = Sample dilution factor.

13.9 For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, the concentration of formaldehyde in sample well is calculated as:

$$HCHO = \left(\frac{(RFUs\ cor)}{(RFUs + Bs\ cor) - (RFUs\ cor)}\right) * HCHO\ spike\ (pmol)$$

Where:

RFUs cor = Fluorescence sample corrected

RFUs = Fluorescence sample

Bs cor = HCHO amount from standard curve corrected

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

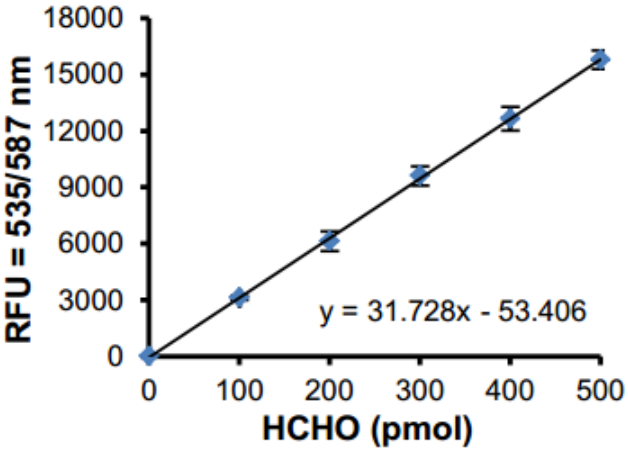


Figure 1. Typical Formaldehyde Standard/HCHO standard calibration curve using fluorometric reading.

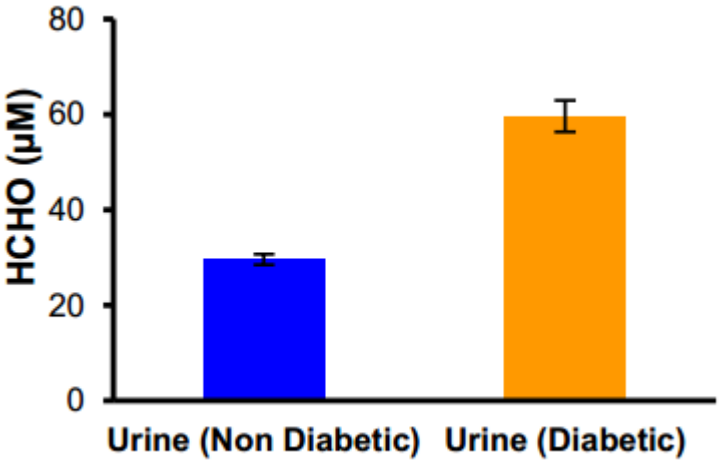


Figure 2: Measurement of Formaldehyde concentration in human urine from non-diabetic and diabetic donors. Samples were deproteinized using 10 kD Spin Column (ab93349) and diluted 10X. Diluted samples (20 µL) were spiked with known amount of HCHO (300 pmol).

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, Formaldehyde Enzyme Mix/enzyme mix, Developer Mix II/developer and PicoProbe I/probe (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL), samples (50 µL) and background wells (50 µL).
- Prepare Formaldehyde Reaction Mix (Number samples + standards + 1).

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
HCHO Assay Buffer/HCHO Assay Buffer	**43**	**45**
Formaldehyde Enzyme Mix/HCHO Enzyme Mix	**2**	**0**
Developer Mix II/HCHO Developer	**2**	**2**
PicoProbe I/PicoProbe	**3**	**3**

- Add 50 µL of Reaction Mix to the standard and sample wells.
- Add 50 µL of Background Mix to the background sample wells.
- Incubate plate at 25°C 20 min protected from light.
- Measure plate at Ex/Em= 535/587 nm for fluorometric assay.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. **FAQ**

18. **INTERFERENCES**

19. **NOTES**

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp