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ab219923 Aldehyde Quantitation Assay Kit II (Colorimetric)

For the rapid, sensitive and accurate measurement of aldehyde in various samples.

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

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1. Overview

Aldehyde Quantification Assay Kit II (Colorimetric) (ab219923) provides a sensitive mix-and-read method to detect aldehyde in a variety of samples such as cell lysates or biological fluids. This assay used a proprietary sensor that upon reacting with an aldehyde, generates a chromogenic product that can be detected by absorbance on a microplate reader at OD 620-660 nm. This assay can detect as little as 3 µM aldehyde (0.3 nmol/100 µL assay volume).

The assay can be performed in a convenient 96-well or 384-well plate format and is easily adapted to automation without a separation step.

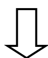
If your plate reader does not have the appropriate filter to detect the absorbance change, we recommend Aldehyde Quantification Assay Kit (Colorimetric) (ab112113), which can be read at OD405 or OD550 nm.

Very reactive aldehydes, namely 4-hydroxyalkenals, were first shown to be formed in autoxidizing chemical systems. It was subsequently shown that 4-hydroxyalkenals, particularly 4-hydroxynonenal, were formed in substantial amounts under biological conditions, i.e. during the peroxidation of lipids of liver microsomes incubated in the NADPH-Fe system. Many other aldehydes were also identified in peroxidizing liver microsomes orhepatocytes, e.g., alkanals, alk-2-enals, and 4-hydroxyalkenals.

Rapid and accurate measurement of aldehydes is an important task for biological and chemical research, as well as food industry and environmental pollution surveillance.

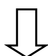
2. Protocol Summary

Standard curve preparation

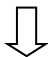


Sample preparation

Add reaction mix and incubate at RT for 20 minutes



Add Blue enhancer



Measure absorbance (OD620 nm) in kinetic mode

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.
- 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.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

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.

Aliquot components in working volumes before storing at the recommended temperature.

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 temperature (before prep)	Storage temperature (after prep)
Assay Buffer	25 mL	-20°C	-20°C
Aldehyde Standard	1 vial	-20°C	-20°C
Blue Dye	2 bottles	-20°C	-20°C
Blue Enhancer	10 mL	-20°C	-20°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at OD 620 nm (range 620-660 nm)
- PBS
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using tissue)

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.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer:

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

9.2 Aldehyde Standard:

Reconstitute the Aldehyde Standard in 1 mL of Assay Buffer to generate a 10 mM aldehyde standard stock solution. Keep on ice while in use. Aliquot standard so that you have enough to perform the desired number of assays. Store at -20°C.

9.3 Blue Dye:

Prepare a **2X Blue Reaction Mixture** by adding 5 mL of Assay Buffer to 1 bottle of Blue dye and mix well.

Δ **Note**: 5 mL of 2X Blue Reaction is enough for one plate. The reaction mixture is not stable, and should be used within 2 hours of preparation.

Store undiluted Blue Dye at -20°C.

9.4 Blue Enhancer:

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

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Using the 10 mM Aldehyde standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard#	Sample to dilute	Volume standard in well (µL)	Assay Buffer (µL)	End conc Aldehyde in well
1	10 mM	5	495	100 µM
2	Std #1	250	250	50 µM
3	Std #2	250	250	25 µM
4	Std #3	250	250	12.5 µM
5	Std #4	250	250	6.25 µM
6	Std #5	250	250	3.125 µM
7	Std #6	250	250	1.56 µM
8 (blank)	0	0	200	0 µM

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 snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- Samples prepared by other protocols can be used as well for this assay. Do not use RIPA buffer as it will interfere with the assay. If you have your samples ready, please skip this section and proceed to Assay Procedure section.

11.1 Cell lysates:

11.1.1 Harvest the number of cells necessary for each assay (initial recommendation: 2-5 x 10^5 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend or scrape cells in 100 µL of cold PBS.

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

11.1.5 Centrifuge 5 minutes at 4°C at 13,000 x*g* in a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a new tube.

11.1.7 Keep on ice.

11.2 Tissue lysates:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation: 20 mg).

11.2.2 Wash tissue with cold PBS.

11.2.3 Homogenize tissue in 400 µL Assay Buffer using a Dounce homogenizer.

11.2.4 Centrifuge homogenate at 2,500 rpm for 5 – 10 minutes at 4°C.

11.2.5 Transfer supernatant to a new tube. Discard pellet.

11.2.6 Keep sample on ice.

11.3 Plasma, Serum and Urine (and other biological fluids):

Samples can be used directly or diluted in Assay Buffer for testing.

Δ **Note**: We suggest using different volumes of sample to ensure readings are within the standard curve range.

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.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

12.1 Reaction wells set up:

- Blank control = 50 µL Assay Buffer.
- Standard wells = 50 µL standard dilutions.
- Sample wells = 1-50 µL samples (adjust volume to 50 µL/well with Assay Buffer).

12.2 Run Aldehyde Assay:

12.2.1 Add 50 µL of 2X Blue Reaction Mixture (Step 9.3) into each well to make the total aldehyde assay volume of 100 µL/well.

12.2.2 Incubate reaction mixture at room temperature for 20-30 minutes protected from light.

12.3 Measurement:

12.3.1 Add 50 µL of Blue Enhancer into each well.

12.3.2 Measure absorbance increase on a microplate reader at OD 620 nm (range 620-660nm, max at 620 nm) in kinetic mode.

Δ **Note:** Incubation time depends on the amount of aldehyde present in the samples. We recommend measuring OD in a kinetic mode, and choosing a time point that fits within the linear range.

13. Protocol for 384-well plate assay

13.1 Prepare standard as described in Section 10.
13.2 Prepare samples as described in Section 11.
13.3 Assay set up: add 12.5 µL standard dilutions and 12.5 µL test samples.
13.4 Add 12.5 µL of 2X Blue Reaction Mixture (Step 9.3) into each well.
13.5 Mix and incubate at room temperature for 20-30 min.
13.6 Add 25 µL Blue Enhancer into each well.
13.7 Measure absorbance increase on a microplate reader at OD 620 nm (range 620-660nm, max at 620 nm) in kinetic mode.

Δ **Note:** Incubation time depends on the amount of aldehyde present in the samples. We recommend measuring OD in a kinetic mode, and choosing a time point that fits within the linear range.

14. Calculations

– Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

14.1 Average the duplicate reading for each standard and sample.
14.2 Subtract the mean absorbance value of the blank (Standard #7) from all standard and sample readings. This is the corrected absorbance.
14.3 Plot the corrected absorbance values for each standard as a function of the final concentration of aldehyde.
14.4 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).
14.5 Apply the corrected sample OD reading to the standard curve to get Aldehyde (B) amount in the sample wells.
14.6 Concentration of aldehyde (nmol/µL or µM) in the test samples is calculated as:

$$Aldehyde\ concentration = \frac{B}{V} * D$$

Where:

B = amount of aldehyde in the sample well calculated from standard curve (nmol).

V = sample volume added in the sample wells (µL).

D = sample dilution factor if sample is diluted to fit within the standard curve range.

15. Typical Data

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

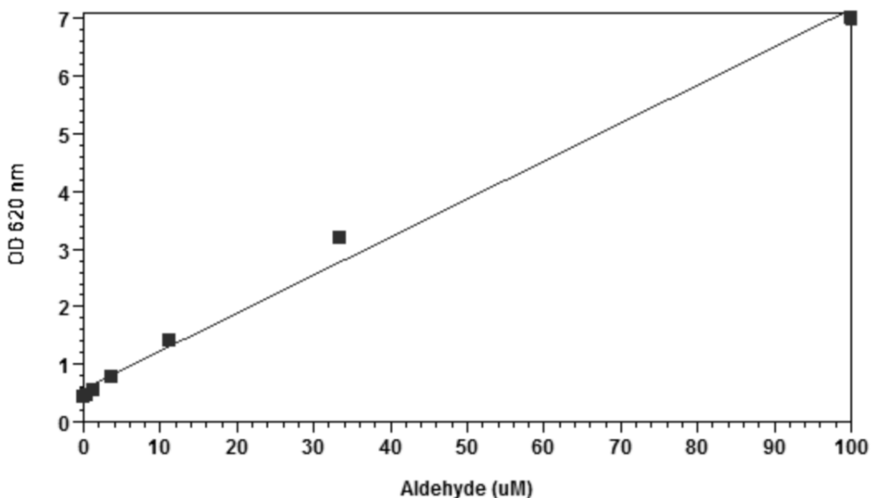


Figure 1. Typical aldehyde standard calibration curve. Aldehyde dose response was measured in a white wall/clear bottom 96-well plate using a SpectraMax microplate reader (Molecular Devices). As low as 3 µM aldehyde can be detected with 30 minutes incubation (n=3).

16. Quick Assay Procedure

Δ 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 reagents and aliquot; get equipment ready.
- Prepare Aldehyde standard dilution [1.56-100 µM].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL) and samples (50 µL).
- Add 50 µL of 2X Blue Reaction Mixture into each well.
- Incubate plate at RT for 20-30 minutes.
- Add 50 µL Blue Dye into each well.
- Measure plate at OD 620 nm.

17. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	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	Reason	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 described in the 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

18. Interferences

These chemical or biological materials will cause interferences in this assay causing compromised results or complete failure:

- BSA (bovine serum albumin): use < 0.001% BSA in samples
- Tween 20: use < 0.01% in samples

19. Notes

Technical Support

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