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ab242295
AOPP Assay Kit

View AOPP Assay Kit datasheet:
www.abcam.com/ab242295

For the quantitative measurement detection of advanced oxidation protein products in plasma, lysates, and tissue homogenates.

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

Table of Contents

1. Overview

AOPP Assay Kit (ab242295) is a simple, reproducible, and consistent system for the detection of advanced oxidation protein products in plasma, lysates, and tissue homogenates. This kit includes a Chloramine standard and an AOPP Human Serum Albumin conjugate for use as a positive control.

Each kit provides sufficient reagents to perform 200 tests including standard curve and unknown samples.

2. Protocol Summary

Prepare standards and samples and add 200 µL to of each to 96-well plate.

Add 10 µL Chloramine Initiator Solution to each well and incubate on shaker for 5 minutes at room temperature.

Add 20 µL of Stop Solution to each well.

Immediately read absorbance at 340 nm.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage condition
Chloramine Standard	20 µL	+4°C
Chloramine Reaction Initiator	1.0 g	+4°C
Stop Solution	5 mL	+4°C
10X Assay Diluent	20 mL	+4°C
AOPP-HSA Positive Control	100 µL	-20°C

5. Materials Required, Not Supplied

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

- Samples for testing
- Microplate reader equipped capable of reading absorbance at 340 nm
- 1X PBS
- Microfuge
- Centrifuge

6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Any components not listed here are ready to use as supplied.

6.1 Chloramine Reaction Initiator:

6.1.1 Weigh out enough AOPP Reaction Initiator for a 200 mg/mL solution. Dissolve the powder in distilled or deionized water.

6.1.2 Prepare only enough for the desired number of tests (e.g. 100 mg dissolved in a final volume of 0.5 mL is enough to run 50 tests).

Δ Note: The Chloramine Reaction Initiator solution is stable for 24-48 hours. Do not store or reuse diluted solutions.

6.2 1X Assay Diluent:

6.2.1 Dilute the 10X Assay Diluent 1:10 with distilled or deionized water.

6.3 AOPP-HSA Positive Control:

6.3.1 Immediately before use, dilute an appropriate amount of the AOPP-HSA Positive Control 1:20 with 1X Assay Diluent.

7. Standard Preparation

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

7.1 Dilute the Chloramine Standard 1:1000 in 1X Assay Diluent for a 100 µM solution. Prepare a dilution series of Chloramine Standard in the concentration range of 100 µM – 0 µM by diluting the 100 µM Chloramine solution in 1X Assay Diluent.

7.2 Prepare a series of the remaining antioxidant standards as shown below:

Standard #	Chloramine Standard (µL)	1X Assay Diluent (µL)	Chloramine Conc (µM)
1	500	0	100
2	400	100	80
3	300	200	60
4	200	300	40
5	100	400	20
6	50	450	10
7	25	475	5
8	0	500	0

8. 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 for the most reproducible assay.

Δ Note: Samples should be prepared at the discretion of the user.

9. 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.

9.1 Prepare samples as desired. Samples such as plasma can be diluted in 1X Assay Diluent or PBS.

9.2 Prepare and mix all reagents thoroughly before use. Each AOPP-containing sample, the AOPPHSA Positive Control, and Chloramine standards should be assayed in duplicate. High content AOPP samples can be further diluted for optimal analysis.

9.3 Add 200 µL of samples or standards to separate wells of the microtiter plate.

9.4 Add 10 µL of Chloramine Reaction Initiator to each well. Mix thoroughly and incubate on a table top rotator or shaker for 5 minutes.

9.5 Add 20 µL of Stop Solution to each well. Mix thoroughly.

9.6 Read the absorbance of each well immediately on a spectrophotometric plate reader using 340 nm as the primary wave length. Use the 0 µM Chloramine Standard as an absorbance blank.

10. Data Analysis

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.

10.1 Calculate the sample AOPP content by reference to the Chloramine Standard curve.

11. Typical Data

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

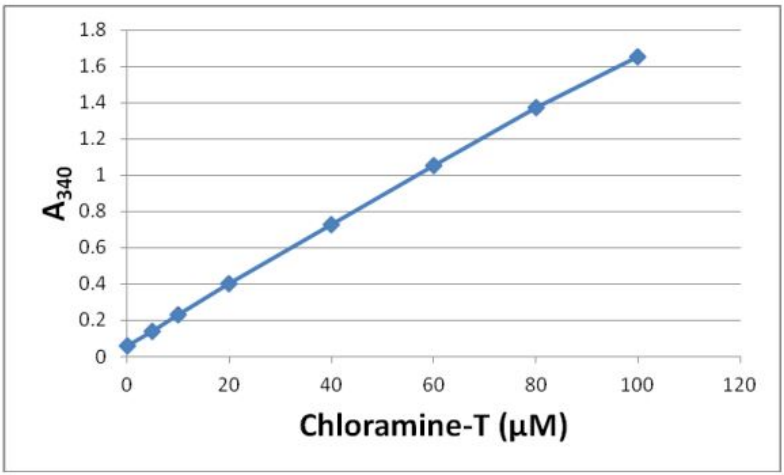

Figure 1. Chloramine standard curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

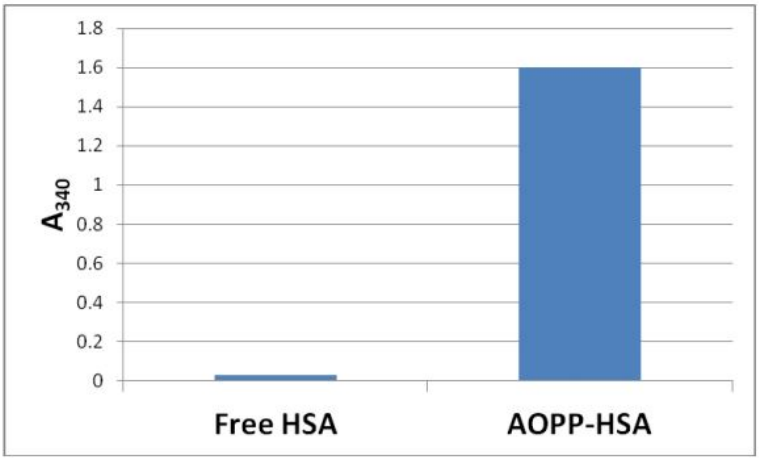

Figure 2. The AOPP-HSA Positive Control and untreated HSA were both prepared at a concentration of 100 µM.

12. Notes

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