Product Manual

OxiSelect™ AOPP Assay Kit

Catalog Number

STA-318 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

Oxidative stress is defined as an increase in the production of reactive oxygen species (ROS) due to an imbalance between antioxidant and oxidants. Advanced Oxidation Protein Products (AOPP) are uremic toxins created during oxidative stress through the reaction of chlorinated oxidants, such as chloramines and hypochlorous acid, with plasma proteins. AOPPs are structurally similar to Advanced Glycation End-Product (AGE) proteins and exert similar biological activities. AOPPs are elevated in patients with renal complications, atherosclerosis, diabetes mellitus, systemic sclerosis, as well as HIV-positive patients. Human Serum Albumin (HSA) treated with HOCl and AOPP generated *in vivo* can ignite oxidative reactions in both neutrophils and monocytes, which indicates both can be used as true mediators of inflammation. Although the mechanisms of AOPP degradation and elimination from the blood remain to be fully elucidated, it appears that the liver and spleen are mostly responsible for their isolation and removal.

The AOPP Assay has provided relevant information concerning free radical activity in many uremic associated disease states and the measurement of anti-oxidant characteristics of many compounds. The rapid and easy protocol has been modified by researchers in the evaluation of plasma and tissue samples. The AOPP-HSA concentration was determined from a Chloramine equivalence standard.

The OxiSelect™ AOPP Assay Kit offers 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.

Assay Principle

The Advanced Oxidation Protein Products (AOPP) Assay Kit is a bioassay tool for the direct quantitative measurement of AOPPs in biological samples. The unknown AOPP-containing samples or Chloramine standards are first mixed with an assay reaction initiator that begins a color development process. After a brief incubation, a stop solution is added and the samples and standards can be read with a standard colorimetric plate reader. The AOPP content in unknown samples is determined by comparison with the predetermined Chloramine standard curve.

Related Products

1. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
2. STA-308: OxiSelect™ Protein Carbonyl Immunoblot Kit
3. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
4. STA-816: OxiSelect™ N-epsilon-(Carboxymethyl) Lysine (CML) Competitive ELISA Kit
5. STA-817: OxiSelect™ Advanced Glycation End Products (AGE) Competitive ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Chloramine Standard (Part No. 231801): One 20 µL tube of 100 mM Chloramine.
2. Chloramine Reaction Initiator (Part No. 231802): One 1.0 g bottle of powder.
3. Stop Solution (Part No. 231803): One 5 mL bottle.
4. 10X Assay Diluent (Part No. 231804): One 20 mL bottle.

Box 2 (shipped on blue ice packs)

1. AOPP-HSA Positive Control (Part No. 231805): One 100 µL tube of 7.5 mg/mL AOPP-Human Serum Albumin at 0.14 µmol AOPP/mg proteins.

Materials Not Supplied

1. Protein samples such as purified protein, plasma, serum, cell lysates
2. Microcentrifuge and conical tubes
3. 96-well Microtiter Plate
4. Centrifuge
5. Container for preparing diluted solutions
6. Adjustable single channel micropipettes with disposable tips
7. Adjustable multichannel micropipette with disposable tips
8. Spectrophotometric microplate reader capable of reading at 340nm
9. 1X PBS

Storage

Upon receipt, store the AOPP-HSA positive control at -20ºC. Store all other components at 4ºC.

Preparation of Reagents

- 1X Assay Diluent: Dilute the 10X Assay Diluent 1:10 with distilled or deionized water.
- Chloramine Reaction Initiator: Weigh out enough AOPP Reaction Initiator for a 200 mg/mL solution. Dissolve the powder in distilled or deionized water. Prepare only enough for the desired number of tests (eg. 100 mg dissolved in a final volume of 0.5 mL is enough to run 50 tests). It is recommended that the AOPP-HSA Positive Control be performed in duplicate each time the assay is used.
  Note: The Chloramine Reaction Initiator solution is stable for 24-48 hours. Do not store or reuse diluted solutions.
- AOPP-HSA Positive Control: Immediately before use, dilute an appropriate amount of the AOPP-HSA Positive Control 1:20 with 1X Assay Diluent.

Preparation of Standard Curve

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 (Table 1). It is recommended that standards be performed in duplicate.

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

Table 1. Preparation of Chloramine Standards

Assay Protocol

1. Prepare samples as desired. Samples such as plasma can be diluted in 1X Assay Diluent or PBS.
2. Prepare and mix all reagents thoroughly before use. Each AOPP-containing sample, the AOPP-HSA Positive Control, and Chloramine standards should be assayed in duplicate. High content AOPP samples can be further diluted for optimal analysis.
3. Add 200 µL of samples or standards to separate wells of the microtiter plate.
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.
5. Add 20 µL of Stop Solution to each well. Mix thoroughly.
6. Read the absorbance of each well immediately on a spectrophotometric plate reader using 340 nm as the primary wave length. Use the 0µM Chloramine standard as an absorbance blank.

Example of Results

The following figures demonstrate typical AOPP Assay results for the Chloramine Standard curve and the AOPP-HSA Positive Control. One should use the data below for reference only. This data should not be used to interpret actual results.

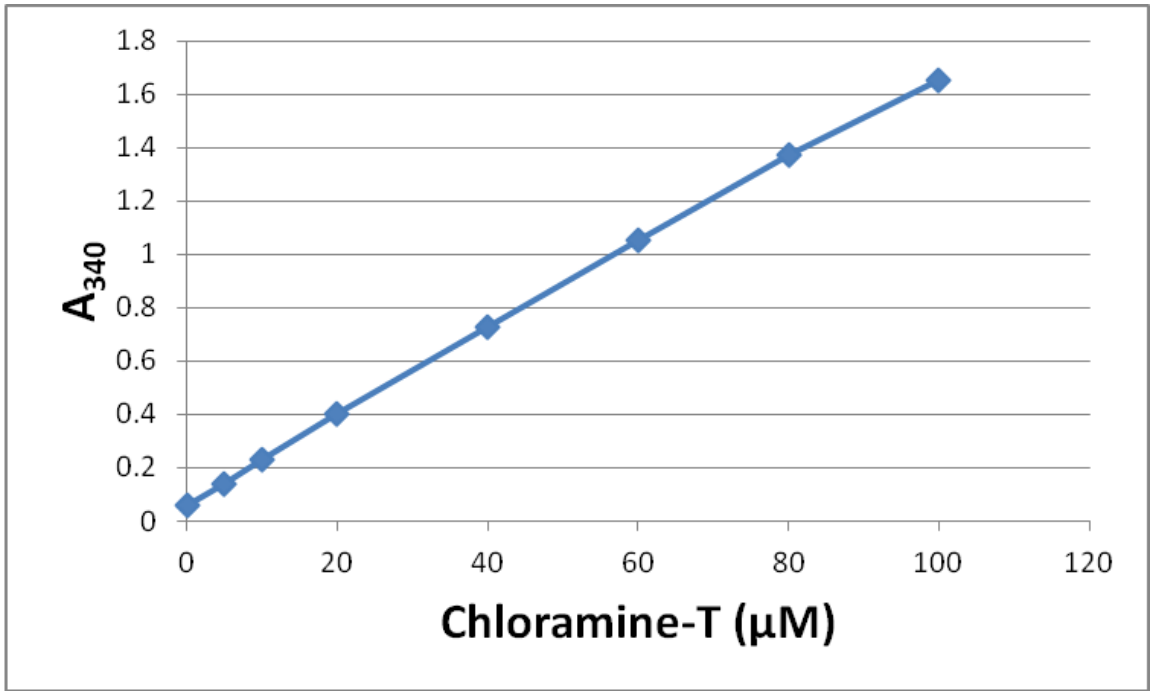

Figure 1. Chloramine Standard Curve for the AOPP Assay. The Chloramine standard curve was created as described in the Assay Protocol.

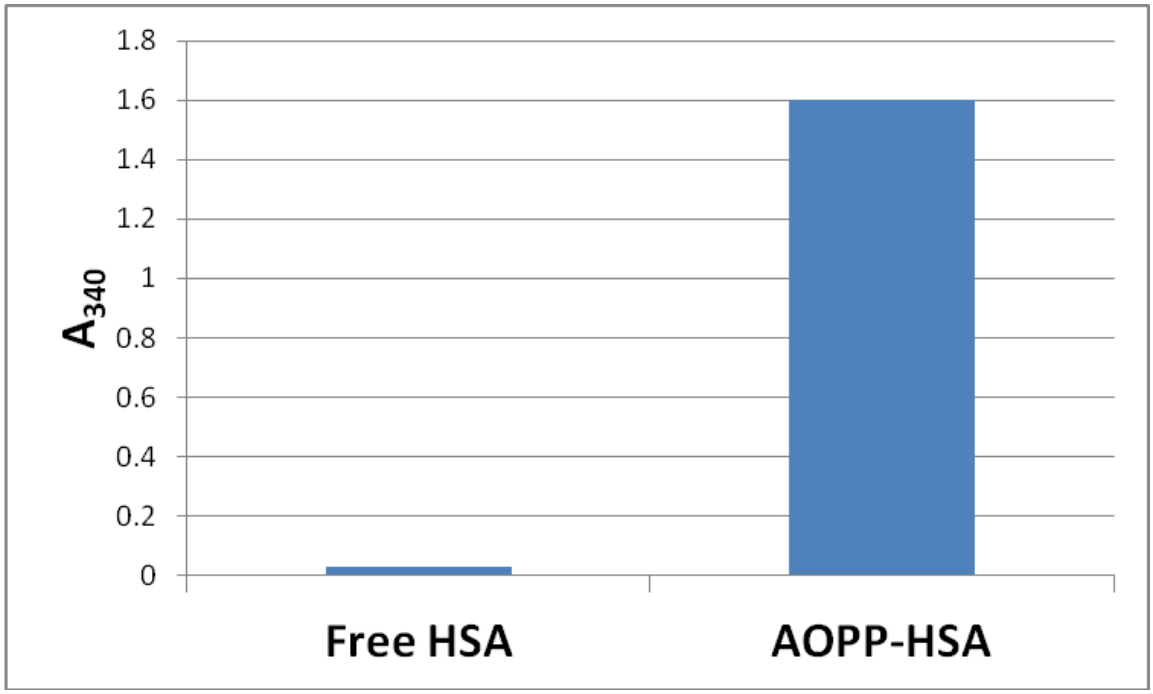

Figure 2. The AOPP-HSA Positive Control and untreated HSA were both prepared at a concentration of 100 µM and tested with STA-318 according to the assay protocol.

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Recent Product Citations

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