CheKine™ Micro Advanced Oxidation Protein Products (AOPP) Assay Kit

Cat #: KTB1060 Size: 100 T/100 S

	Micro Advanced Oxidation Protein Products (AOPP) Assay Kit		
REF	**Cat #:** KTB1060	LOT	**Lot #:** Refer to product label
	Detection range: 5-100 μmol/L		**Sensitivity:** 2.5 μmol/L
	Applicable samples: Cells, Animal and Plant Tissues, Serum, Plasma		
	Storage: Stored for 6 months at -20℃, protected from light		

Assay Principle

Advanced Oxidized Protein Products (AOPP) is a newly discovered class of uremic toxins with pro-inflammatory activity, and is an oxidative modification product formed after plasma proteins are attacked by reactive oxygen species (ROS). AOPP can stimulate monocytes and neutrophils, stimulate the synthesis and release of a large number of pro-inflammatory cytokines, mainly tumor necrosis factor TNF-α, and promote the production of more reactive oxygen species, thereby inducing and aggravating systemic oxidative stress and inflammation. AOPP is very similar to Advanced Glycation End-Product (AGE) proteins, and they also perform similar biological functions. AOPP is an important detection index for diseases such as renal complications, atherosclerosis, diabetes, metabolic syndrome, immune diseases and malignant tumors. CheKine™ Micro Advanced Oxidized Protein Products (AOPP) Assay Kit can quantitatively detect the content of AOPP in samples such as plasma, serum, animal/plant tissues and cells. The sample or standard reacts with the initiator and stop solution, and the product has an absorption peak at 340 nm. By detecting the OD value at 340 nm, and comparing with the standard curve, the content of AOPP in the sample is determined.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
	96 T	
Extraction Buffer (10×)	15 mL	4℃
Reagent Ⅰ	1.5 mL	4℃, protected from light
Reagent Ⅱ	3 mL	4℃
Standard	1 mL	4℃, protected from light
AOPP-HSA Positive Control	50 μL	-20℃
Free-HSA Negative Control	50 μL	-20℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Ice maker, refrigerated centrifuge, incubator
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer (1×): prepared before use, diluted 1:9 to 1× with deionized water, and mix well for use.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Note: Reagent Ⅱ or Standard has certain irritation, so personal protection is recommended during use.

Working AOPP-HSA Positive Control: Prepared before use, diluted 1:19 with Extraction Buffer (1×), and mixed for use. The remaining reagent can be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Working Free-HSA Negative Control: Prepared before use, diluted 1:19 with Extraction Buffer (1×), and mixed for use. The remaining reagent can be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Standard curve setting: Dilute 1,000 μmol/L Standard with deionized water to 100, 80, 60, 40, 20, 10, 5 μmol/L standard solution as shown in the table below.

Num.	Volume of 1,000 μmol/L Standard (μL)	Volume of Deionized Water (μL)	The Concentration of Standard (μmol/L)
Std.1	100	900	100
Std.2	80	920	80
Std.3	60	940	60
Std.4	40	960	40
Std.5	20	980	20
Std.6	10	990	10
Std.7	5	995	5
Std.8	0	1,000	0

Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

1. Cell: Collect 5×10^6 cells into the centrifuge tube, and discard the supernatant after centrifugation; add 1 mL Extraction Buffer (1×) to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 13,000 g for 10 min at 4℃. Use supernatant for assay.

2. Tissue samples: Weigh 0.1 g tissue, and add 1 mL Extraction Buffer (1×) and homogenize. Centrifuge at 13,000 g for 10 min at 4℃. Use supernatant for assay.

3. Serum and plasma: Dilute 5 times with Extraction Buffer (1×) and detect directly.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the

content is calculated by protein concentration.

Assay Procedure

1. Preheated the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Add the following reagents respectively into each 96-well UV plate or microquartz cuvette:

Reagent	Test Well (μL)	Blank Well (μL)	Standard Well (μL)	Positive Control Well (optional) (μL)	Negative Control Well (optional) (μL)
Sample	200	0	0	0	0
Extraction Buffer (1×)	0	200	0	0	0
Working AOPP-HSA Positive Control	0	0	0	200	0
Working Free-HSA Negative Control	0	0	0	0	200
Standard	0	0	200	0	0
Reagent Ⅰ	10	10	10	10	10
Reagent Ⅱ	20	20	20	20	20
Mix well, then incubate 5 min at 37 ℃ . And reading the values at 340 nm. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$, $\Delta A_{Positive\ Control}=A_{Positive\ Control}-A_{Blank}$, $\Delta A_{Negative\ Control}=A_{Negative\ Control}-A_{Blank}$.					

Note: The Control Well, the Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.002, increase the sample quantity appropriately. If ΔA_{Test} is larger than 100 μmol/L of $\Delta A_{Standard}$, the sample can be appropriately diluted with Extraction Buffer (1×), the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the y-axis and $\Delta A_{Standard}$ as the x-axis, draw the standard curve. Substitute the ΔA_{Test} into the equation to obtain the y value (μmoL/L).

2. Calculate the content of AOPP in sample

(1) By protein concentration

AOPP content (μmol/g)=$(y\times V_{sample})\div(V_{sample}\times Cpr)\times n$**=y÷Cpr×n**

(2) By sample fresh weight

AOPP content (μmol/g)=$(y\times V_{sample})\div(W\times V_{sample}\div V_{Extraction\ Buffer})\times n$**=y÷W÷1,000×n**

(3) By serum and plasma

AOPP content (μmol/L)**=y×n**

(4) By number of Cells

AOPP content (nmol/10^4 cells)=$(y\times V_{sample})\div(500\times V_{sample}\div V_{Extraction\ Buffer})$**=y÷500**

Where: V_{sample}: Added sample volume, 0.2 mL; $V_{Extraction\ Buffer}$: Added Extraction Buffer, 1 mL; Cpr: Protein concentration of the sample, mg/mL; W: Weight of sample, g; 500: number of cells, 5 million; n: Sample dilution factor.

Typical Data

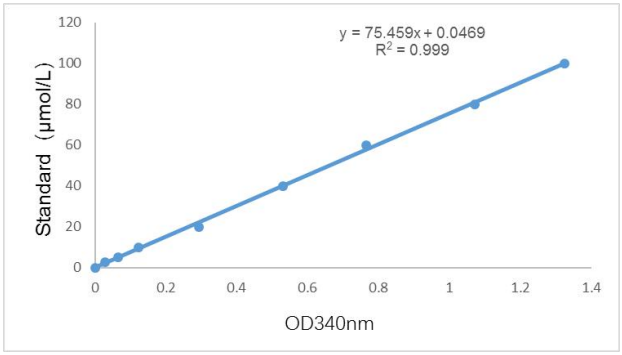

Figure 1. Standard Curve for AOPP.

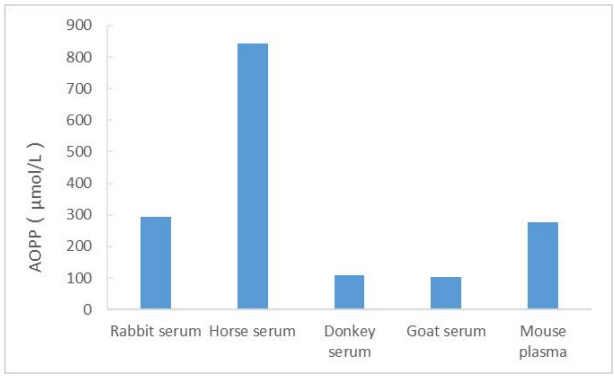

Figure 2. AOPP content in rabbit serum, horse serum, donkey serum, goat serum and mouse plasma respectively. Assays were performed following kit protocol.

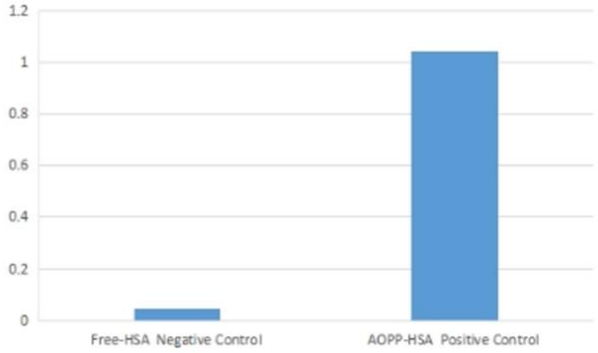

Figure 3. OD values of Free-HSA Negative Control and AOPP-HSA Positive Control at 340 nm.

Recommended Products

Catalog No.	Product Name
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Activity Assay Kit
KTB1910	CheKine™ Reactive Oxygen Species (ROS) Detection Fluorometric Assay Kit
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1500	CheKine™ Micro Total Antioxidant Capacity (TAC) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.