KAMIYA BIOMEDICAL COMPANY

Total Antioxidant Capacity (PAO) Assay

For the assessment of total antioxidants of serum, foods and beverage samples

Cat. No. KT-521

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

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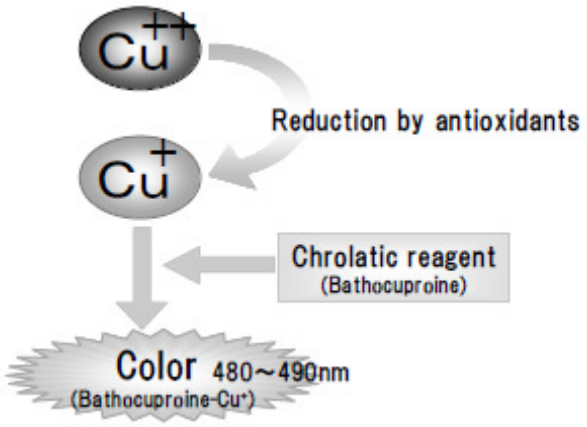
BACKGROUND

Oxidative stress plays an important role in various diseases and aging. The control of oxidative stress is expected to be useful to prevent diseases and aging. Oxidative stress is caused by the imbalance between reactive oxygen species (ROS) and antioxidant defense system. For accurate assessment of oxidative stress, measurement of ROS, oxidative damage and antioxidant activity may be essential. PAO can detect not only hydrophilic antioxidants such as Vitamin C and glutathione, but can also detect hydrophobic antioxidants such as Vitamin E. Applicable for the assessment of total antioxidants of serum, foods, and beverage samples. For research use only.

PRINCIPLE



Samples are mixed with Cu++ Solution. Cu++ are reduced by antioxidants to form Cu+. Reduced Cu+ react with Chromatic Solution (Bathocuproine), and can be detected by absorbance at wavelength 480 to 490 nm. Antioxidant capacity can be calculated from the Cu+ formed.

COMPONENTS

1. Calibrator (Uric acid powder): 1 vial (for 2 mM), Dissolve with distilled water.
2. Sample diluent: 60 mL x 1 bottle, Ready to use.
3. Cu++ solution: 5 mL x 1 bottle, Ready to use.
4. Stop solution: 5 mL x 1 bottle, Ready to use.
5. Microtiter plate: 1 plate

Materials or Equipment required but not provided

A. A microplate reader (measuring wavelength 490 nm)
B. Pipettes and pipette chips
C. Plastic test tubes
D. Distilled water
E. NaOH, HCl solution and pH meter (Not required if calibrators are prepared with distilled water only.)

PROCEDURE

1. Reconstitution of Calibrator (2 mM Uric acid solution): There are two ways for preparation. Please select one.

    a) Add distilled water to the calibrator vial, and let stand for 3 or 4 hours at room temperature. The volume of distilled water is indicated on the label of the vial.

    b) If you wish to prepare calibrator solution immediately, please pour 1 mL of 10% (w/v) NaOH to the calibrator vial, and dissolve completely, followed by pH adjustment (pH 7.4) by HCl solution. Add distilled water to make the total volume as indicated on the label.

2 mM uric acid solution can be stored at below -70℃ for 1 year.

2. Preparation of Calibrators: Dilute 2 mM uric acid solution with distilled water for 2, 4, 8, 16, and 32 times, resulting in 5 levels of diluted calibrators (1 mM, 0.5 mM, 0.25 mM, 0.125 mM and 0.063 mM respectively).

3. Preparation of samples: If you measure serum samples, fresh frozen samples are recommended. Because some antioxidants such as vitamin C, uric acid and coenzyme Q10 are unstable. For other samples such as beverages, see “Assay examples” and dilute with distilled water.

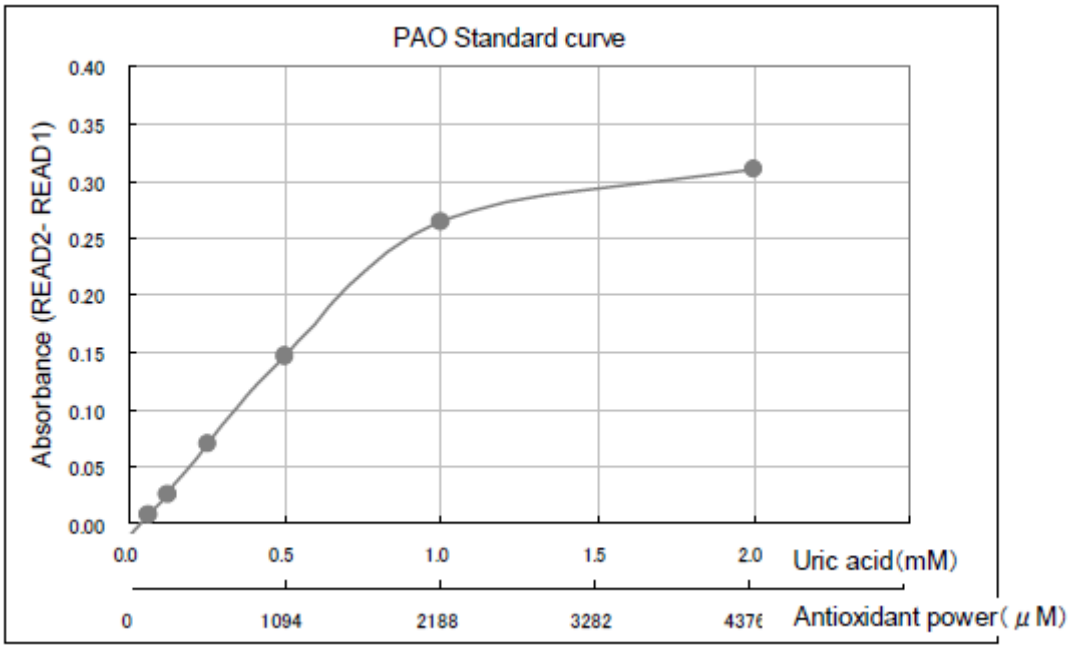
4. Assay procedure:
    a) Please prepare plastic test tubes for 6 levels of calibrators and each sample. Pour 390 µL of sample diluent and add 10 µL of calibrators or diluted samples.
    b) Pour 200 µL of mixture to microtiter plate. Use 200 µL of sample diluent for blank well.
    c) Read absorbance at 490 nm (as READ1).
    d) Add 50 µL of Cu++ solution to each well, mix gently, and incubate at room temperature for 3 minutes.
    e) Add 50 µL of stop solution, mix gently, and read absorbance at 490 nm (as READ2).

5. Determination of antioxidant power of samples: Please draw calibration curves by plotting the difference of absorbance readings (READ2 – READ1) as vertical axis, and concentration of uric acid calibrators (mM) as horizontal axis. Calculate the corresponding uric acid concentration of samples. Multiply corresponding uric acid concentration (mM) of samples by 2189, to estimate antioxidant power (µmol/L).

1 mM of uric acid = 2189 µmol/L (copper reducing power)

CALCULATION OF RESULTS

A typical calibration curve is shown below. Do not use this curve to determine results.



STORAGE

Store at room temperature until the expiration date listed on the kit box label.

ASSAY RANGE

21.9 – 4378 µmol/L (Cupric ion reducing power)

ASSAY EXAMPLES

D.W.: distilled water.

Sample	Pre-dilution	Antioxidant power (μ mol/L)	
Human serum	Not required	1069±145	Fresh frozen serum.
Human urine	Mix with 3 volumes of D.W.	5508	
Red wine	Mix with 7 volumes of D.W.	45479	
Japanese SAKE (rice wine)	Not required	18～211	
Black tea	Mix with 7 volumes of D.W.		
Coffee	Mix with 27 volumes of D.W.		
Green tea	Mix with 7 volumes of D.W.	8728～46687	Green tea products.

A greater dilution is recommended if the antioxidant power is over 2000 µmol/L antioxidant power. For example, some green tea products which contain high concentration of catechin should be diluted by 40 times (mix 1 volume of sample and 39 volumes of distilled water). Some samples which contain chelating agents such as EDTA can't be applied.

FOR RESEARCH USE ONLY

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