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Inspiration for Life Science

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IM-KPA090121E

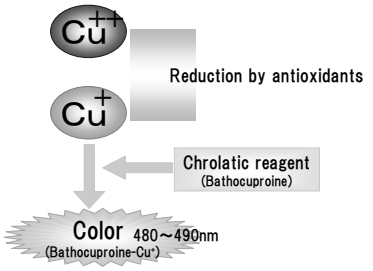
Catalog #NNS-KPA-050E-EX

# PAO Test kit for Total Antioxidant Capacity

# Instruction manual

Oxidative stress plays on 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, glutathione, but also can detect hydrophobic antioxidants such as Vitamin E. Applicable for assessment of total antioxidants of serum, foods and beverage samples.

## 1. Principles and components




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.

| | | |
|---|---|---|
| ①Standard（Uric acid powder） | 1 vial (for 2mM) | Dissolve with distilled water. |
| ②Sample diluent | 60 mL x 1 bottle | Ready to use. |
| ③$Cu^{++}$ solution | 5 mL x 1 bottle | Ready to use. |
| ④Stop solution | 5 mL x 1 bottle | Ready to use. |
| ⑤Micro titer plate | 1 plate | |

## 2. Specifications

①Assay range: 21.9～4378 μmol/L（Cupric ion reducing power）
②Storage: Room temperature
③Expiry date: 3 years（Indicated on the outer box）

## 3. Required but not provided

①A micro plate reader (measuring wavelength 490 nm)
②Pipettes and pipette chips
③Plastic test tubes
④Distilled water
⑤NaOH, HCl solution and pH meter (Not required if standards are prepared with distilled water only)

## 4. Assay procedure

**1) Reconstitute of Standard (2mM Uric acid solution).** There are two ways for preparation. Please select one.

Case 1：Add distilled water to *①Standard vial*, and stand for 3 or 4 hours at room temperature. The volume of distilled water is indicated on the label of the vial.

Case 2: If you wish to prepare standard solution immediately, please pour 1mL of 10% (w/v) NaOH to *①Standard vial*, and dissolve completely, followed by pH adjustment (pH7.4) by HCl solution. Add distilled water to make the total volume as indicated on the label.

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

**2) Preparation of standards.**

Dilute 2mM uric acid solution with distilled water for 2, 4, 8, 16 and 32 times, result in 5 levels of diluted standards (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 section 6) Assay examples, and dilute with distilled water.

**4) Assay procedure.**

A) Please prepare plastic test tubes for 6 levels of standards and each sample. Pour 390 μL of *②Sample Diluent*, and add 10 μL of standards or diluted samples.
B) Pour 200 μL of mixture to *⑤Micro titer plate*. Use 200 μL of *②Sample Diluent* for blank well.
C) Read absorbance at 490 nm (as *READ1*).
D) Add 50 μL of *③$Cu^{++}$ solution* to each well, mix gently, and incubate at room temperature for 3 minutes.
E) Add 50 μL of *④Stop solution*, mix gently, and read absorbance at 490 nm (as *READ2*).
(Optional: reference wave length 620 or 660 nm)

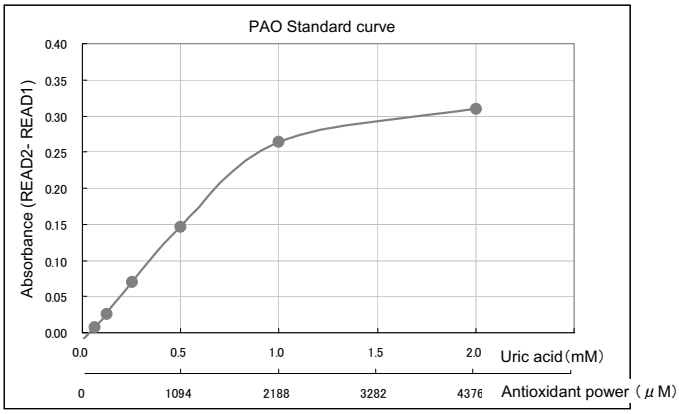
**(Continue to the next page)**

**5) Determination of antioxidant power of samples.**

Please draw standard curves by plotting the difference of absorbance readings (*READ2 - READ1)* as vertical axis, and concentration of uric acid standards (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 ( $\mu$ mol/L).

**1mM of uric acid = 2189 $\mu$ mol/L (copper reducing power)**

## 5. Typical standard curves




## 6. Assay examples

D.W. : distilled water.

| Sample | Pre-dilution | Antioxidant power ( $\mu$ 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 more dilution is recommended if the antioxidant power is over 2000 $\mu$ mol/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 volume of distilled water). Some samples which contain chelating agents such as EDTA can't be applied.

## 7. References

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For more information and technical support, please visit CosmoBio web site **http://www.cosmobio.co.jp/**

The "PAO assay kits" are inventions by Dr. Giuseppe Da Cruz, Italy (US PAT. #6613577 and IT PAT. #1309421).



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