Antioxidant Capacity Potential Assay

Catalog Number: PAC39-K01
96 Wells
For Research Use Only
v. 1.0

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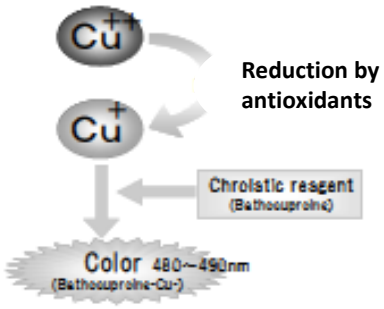
Intended Use:

The Eagle Biosciences Antioxidant Capacity Potential Assay kit is intended for the quantitative determination of antioxidant capacity in biological samples as well as well food and beverage samples by enzyme linked immunoassay (ELISA). The Antioxidant Capacity Potential Assay kit is for research use only and not to be used in diagnostic procedures.

Assay Background:

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. The Eagle Biosciences Potential Antioxidant Capacity Assay can detect not only hydrophilic antioxidants such as Vitamin C and glutathione, but also can detect hydrophobic antioxidants such as Vitamin E. This Antioxidant Capacity Potential Assay kit is applicable for assessment of total antioxidants of serum, foods and beverage samples.

Principle of Procedure:



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.

Materials Provided with the Assay:

• Standard (Uric acid powder)	1 vial (for 2mM)	Dissolve with distilled water.
• Sample diluent	60 mL x 1 bottle	Ready to use.
• Cu ++ solution	5 mL x 1 bottle	Ready to use.
• Stop solution	5 mL x 1 bottle	Ready to use.
• Micro titer plate	1 plate	

Materials Required but not Provided:

- Microplate reader (measuring wavelength 490 nm)
- Pipettes and pipette tips
- Plastic test tubes
- Distilled water
- NaOH, HCl solution and pH meter (Not required if standards are prepared with distilled water only)

Specifications:

- Assay range: 21.9～4378 μmol/L （Cupric ion reducing power）
- Storage of the Antioxidant Capacity Potential Assay: Room Temperature
- Expiration date: 3 years from manufacture date

Assay Procedure

1. Reconstitute of Standard (2mM Uric acid solution).
    - There are two options for preparation. Select one.
        i. Option 1: Add distilled water to the Standard vial, and stand for 3 or 4 hours at room temperature. The volume of distilled water is indicated on the label of the vial.
        ii. Option 2: If you wish to prepare standard solution immediately, add 1mL of 10% (w/v) NaOH to Standard 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°C 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.
    - For serum samples, fresh frozen samples are recommended because some antioxidants such as vitamin C, uric acid and coenzyme Q10 are unstable. For other samples types such as beverages and food, see section "Assay Examples", and dilute with distilled water.

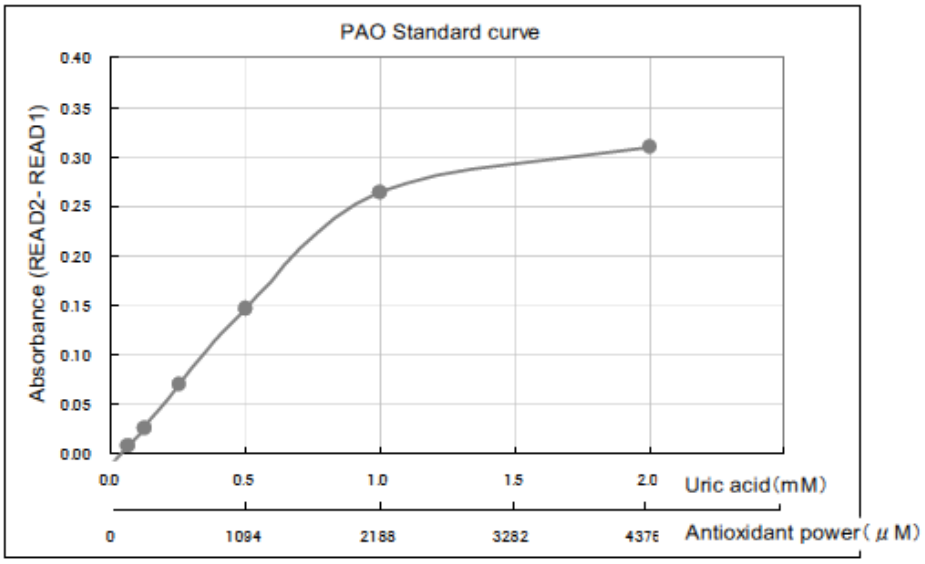
4. Assay procedure.
    1. Please prepare plastic test tubes for 6 levels of standards and each sample. Pour 390 µL of Sample Diluent, and add 10 µL of standards or diluted samples.
    2. Pour 200µL of mixture to Micro titer plate. Use 200µL of Sample Diluent for blank well.
    3. Read absorbance at 490 nm (as READ1).
    4. Add 50µL of Cu++ solution to each well, mix gently, and incubate at room temperature for 3 minutes.
    5. Add 50µL of Stop solution, mix gently, and read absorbance at 490 nm (as READ2).

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 (µmol/L).

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

Typical Standard Curve



Assay Examples:

Sample	Pre-dilution	Antioxidant power (μmol/L)
Human serum	Not required	1069±145
Human urine	Mix with 3 volumes of Distilled Water	5508
Red wine	Mix with 7 volumes of Distilled Water	45479
Japanese SAKE (rice wine)	Not required	18~211
Black tea	Mix with 7 volumes of Distilled Water	TBD
Coffee	Mix with 27 volumes of Distilled Water	TBD
Green tea	Mix with 7 volumes of Distilled Water	8728~46687

Additional 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 volume of distilled water). Some samples which contain chelating agents such as EDTA can't be applied with this Antioxidant Capacity Potential Assay.

References:

- Oxidative stress and its association with coronary artery disease and different atherogenic risk factors C. VASSALLE, L. PETROZZI , N. BOTTO, M. G. ANDREASSI & G. C. ZUCCHELLI Journal of Internal Medicine 256, p308–315 (2004)
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- Restored Antioxidant Capacity Parallels the Immunologic and Virologic Improvement in Children with Perinatal Human Immunodeficiency Virus Infection Receiving Highly Active Antiretroviral Therapy.
- M Martino, F Chiarelli, M Moriondo, M Torello, C Azzari, and L Galli. Clinical Immunology, Vol.100(1),p82-6 (2001)
- Antioxidant capacity as a reliable marker of stress in dairy calves transported by road. P Preget, E Bollo, FT Cannizzo, B Biolatti, E Contato, PG Biolatti: Veterinary Record Vol. 156, p53-54 (2005)

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