EZMeta™ Hydroxyl Radical Antioxidant Capacity (HORAC) Fluorometric Assay Kit

Cat #: D-AKC1502

Size: 48T / 96T

Storage: Stored at -20℃ for 12 months, protected from light

Product Information

Detection range: 1.25-40 μM

Sensitivity: 1.25 μM

Applicable samples: Animal and Plant Tissues, Cells, Bacteria, Serum, Plasma

Assay Principle

Hydroxyl Radical Absorbance Capacity (HORAC) is a method to detect the antioxidant Capacity of biological molecules in various samples, which can reflect the ability of antioxidants to transfer Hydroxyl free radicals. EZMeta™ Hydroxyl Radical Antioxidant Capacity (HORAC) Fluorometric Assay Kit can detect animal and plant tissues, cells, bacteria, serum, plasma and other samples. The principle is based on fluorescein sodium fluorescence probe, the fenton reaction produces hydroxyl free radicals, according to hydroxyl free radical damage fluorescent probe. When the fluorescence intensity changes, the change of fluorescence intensity reflects the degree of free radical damage. In the presence of antioxidants, it inhibits fluorescence changes caused by free radicals. The degree of inhibition reflects its antioxidant ability to free radicals.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Assay Buffer (2×)	50 mL	100 mL	4°C
Reagent Ⅰ (100×)	100 µL	200 µL	-20°C, protected from light
Reagent Ⅱ (32×)	50 µL	100 µL	4°C, protected from light
Reagent Ⅲ	1.5 mL	3 mL	-20°C, protected from light

Materials Required but Not Supplied

·Fluorescence microplate reader (the excitation wavelength is 485 nm, and the emission wavelength is 525 nm)

·Black 96-well plate, precision pipettes, disposable pipette tips

·Refrigerated centrifuge, incubator, ice maker

·Deionized water, PBS (pH 7.4), acetone

·Dounce homogenizer (for tissue samples)

Reagent Preparation

1×Assay Buffer: Before use, dilute Assay Buffer (2×) to 1×Assay Buffer with deionized water and fully dissolve. Store at 4°C.

1×Reagent I: Before use, dilute Reagent Ⅰ (100×) to 1×Reagent Ⅰ with 1×Assay Buffer and fully dissolve. The remaining Reagent Ⅰ (100×) can also be stored at -20°C and protected from light after aliquoting to avoid repeated freezing and thawing.

Note: Do not store the diluted 1×Reagent Ⅰ solution.

1×Reagent Ⅱ: Before use, dilute Reagent Ⅱ (32×) to 1×Reagent Ⅱ with 1×Assay Buffer and fully dissolve. Store at 4°C, protected from light.

Reagent Ⅲ: Ready to use as supplied. Equilibrate to room temperature before use. The remaining reagent can be stored at -20°C and protected from light. The Reagent Ⅱ reagent is unstable and should be used immediately.

Trolox Standard (5 mM): Before use, dilute with 1×Assay Buffer to 0.1 mM concentration and fully dissolve. The remaining Trolox Standard (5 mM) is stored in aliquots at -20°C and protected from light to avoid repeated freezing and thawing.

Standard setting: Prepare the standard solution as shown in the table below.

Num.	Volume of 0.1 mM Standard(μL)	Volume of 1×Assay Buffer(μL)	Standard Concentration(μL)
Std.1	80	120	40
Std.2	40	160	20
Std.3	20	180	10
Std.4	10	190	5
Std.5	5	195	2.5
Std.6	2.5	197.5	1.25
Std.7	0	200	0

Note: The diluted standard solution is prepared and immediate used, and should not be stored for a long time.

Sample Preparation

Note: Fresh samples are recommended. If not assayed immediately, samples can be stored at -80°C.

1. Animal tissues: Weigh 0.1 g tissue, add an appropriate amount of 1×Assay Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

2. Plant tissues: Weigh 0.1 g tissue, add an appropriate amount of 1×Assay Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

3. Cells or bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add an appropriate amount of 1×Assay Buffer to ultrasonically disrupt the cells or

bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

4. Plasma and serum: Diluted 10 times or more with 1×Assay Buffer to be tested.

5. Liquid sample: Centrifuge at 10,000 g for 10 min at 4°C to remove particles. Use supernatant for assay, and place it on ice. Dilute the supernatant to be tested as required.

6. Lipophilic sample: Lipophilic sample was dissolved in 100% acetone, diluted with 50% acetone, incubated at room temperature for 1 h. Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice. Dilute the supernatant to be tested as required.

7. Solid or high protein sample: weigh the solid sample, add deionized water (1:2, W/V), ice bath homogenate. Centrifuge at 10,000 g for 10 min at 4°C, take the supernatant of the water-soluble part. The insoluble portion was washed with deionized water and mixed with water-soluble supernatant. The combined supernatant could be diluted with 1×Assay Buffer and used for analysis directly. The insoluble part was further extracted by adding pure acetone (1:4, W/V) and mixing at room temperature for 30-60 min. Centrifuged at 10,000 g at 4°C for 10 min, the supernatant of the acetone extract was diluted with 50% acetone. The total HORAC value was calculated by combining the results of acetone extracts from the water-soluble and insoluble fractions.

Assay Procedure

1. Preheat the fluorescence microplate reader to 37°C. The excitation wavelength is 485 nm, and the emission wavelength is 525 nm.

2. Sample measurement (The following operations are operated in the black 96-well plate).

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
1×Assay Buffer	20	0	0
Standard	0	20	0
Supernatant	0	0	20
1×Reagent Ⅰ	140	140	140
Mix well and incubated for 37°C for 30 min			
1×Reagent Ⅱ	20	20	20
ReagentⅢ	20	20	20

3. Mix well and immediately read the fluorescence value with a fluorescence microplate reader, every 5 min once, total time is 60 min. The measurement condition of the fluorescence microplate reader is that the excitation wavelength is 485 nm, the emission wavelength is 525 nm, and the temperature of the instrument is kept at 37°C.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. The final measured value of the blank Well should be less than 10% of the initial value.

Data Analysis

The HORAC values were calculated for antioxidant activity based on the net area under the fluorescence attenuation curve (Area Under the Curve, AUC)=[AUC(sample)-AUC(blank)].

1. Calculate AUC from the following equation

$AUC=1+RFU_1/RFU_0+RFU_2/RFU_0+RFU_3/RFU_0+\ldots\ldots+RFU_{59}/RFU_0+RFU_{60}/RFU_0$

RFU_0=0 min relative fluorescence value.

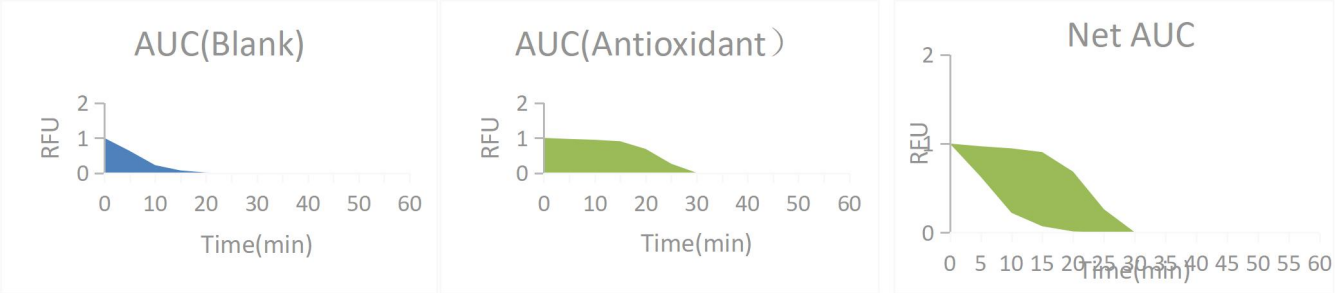
RFU_x=x min relative fluorescence value (for example, RFU_5 is the relative fluorescence value at 5 min).

2. Calculate the Net AUC: Net AUC=AUC (sample)-AUC (blank).

3. Drawing the standard curve: the standard concentration as the y-axis and Net AUC as the x-axis, draw the standard curve.

4. Put the Net AUC of the sample into the equation to obtain the y value (μM), and express the HORAC value in micromole per liter of Trolox equivalent (TE) or TE per g of sample (μmol TE/g or μmol TE/L).

Note: If the sample is further diluted, it needs to be multiplied by the dilution factor n.



Typical Data

Typical standard curve-data:

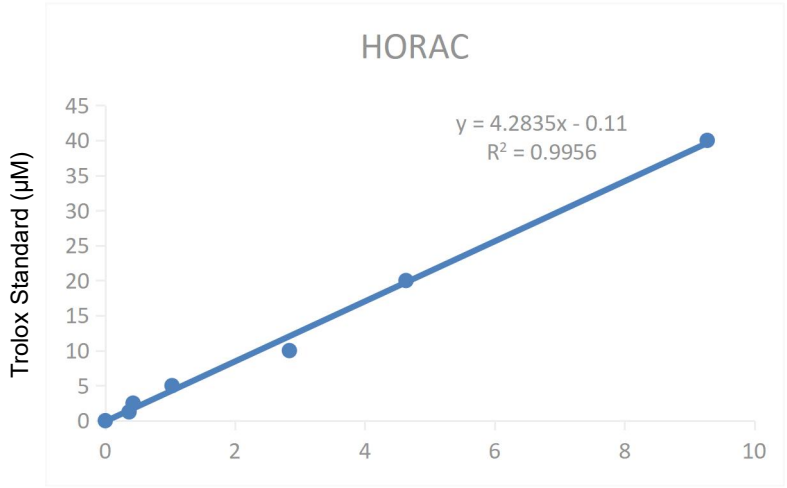


Figure 1. Standard Curve for HORAC.

Example:

Take 0.1 g plant tissue and add 1 mL 1×Assay Buffer to homogenize and grind, take the supernatant, and then follow the measurement procedure, and measure with a black 96-well plate:

The AUC of the sample is 9.199, the blank AUC is 1.937, the Net AUC=AUC(sample)-AUC(blank)=9.199-1.937=7.262, the standard curve is y=4.2835x-0.11, the Trolox concentration is 30.997 μM, the HORAC value(sample)=30.997 μM=30.997 μM TE=30.997 μmol TE/L.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.