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CheKine™ Oxygen Radical Antioxidant Capacity (ORAC) Fluorometric Assay Kit

Cat #: KTB1501 Size: 48 T/48 S 96 T/96 S

	Oxygen Radical Antioxidant Capacity (ORAC) Fluorometric Assay Kit		
REF	**Cat #:** KTB1501	LOT	**Lot #:** Refer to product label
	Detection range: 2.5-50 μM		**Sensitivity:** 2.5 μM
	Applicable samples: Animal and Plant Tissues, Cells, Bacteria, Serum, Plasma		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Oxygen Radical Absorbance Capacity (ORAC) is a classical method to detect the antioxidant capacity of biomolecules in various samples. ORAC method has the advantages of good specificity, high sensitivity, wide determination range and high throughput screening for antioxidant activity. It has been applied more and more in natural product antioxidant extracts. Food and functional food enterprises generally adopt ORAC as an important evaluation standard for functional food. CheKine™ Oxygen Radical Antioxidant Capacity (ORAC) 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, azo compounds AAPH as a source of oxygen free radicals, according to the free radicals damage the 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℃
Reagent Ⅰ (100×)	110 μL	220 μL	-20℃, protected from light
Reagent Ⅱ (0.08 g)	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Trolox Standard (5 mM)	0.5 mL	1 mL	-20℃, protected from light

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

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
- Refrigerated centrifuge, incubator
- Ice maker

- Precision pipettes, disposable pipette tips
- Deionized water, 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℃.

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℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing. **Do not store the diluted 1×Reagent Ⅰ solution.**

Working Reagent Ⅱ : Before use, Prepare 19 mg/mL Reagent Ⅱ solution fresh. For example, weigh 19 mg Reagent Ⅱ and add 1 mL 1×Assay Buffer to fully dissolve. Working Reagent Ⅱ reagent is unstable and should be used immediately.

Trolox Standard (5 mM): Before use, dilute with 1×Assay Buffer to 0.2 mM concentration and fully dissolve. The remaining Trolox Standard (5 mM) is stored in aliquots at -20℃ 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 0.2 mM Standard (μL)	Volume of 1×Assay Buffer (μL)	Standard Concentration (μM)
Std.1	50	150	50
Std.2	40	160	40
Std.3	30	170	30
Std.4	20	180	20
Std.5	10	190	10
Std.6	5	195	5
Std.7	2.5	197.5	2.5
Std.8	0	200	0

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

Sample Preparation

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

1. Animal tissues: Weigh 0.01-0.1 g tissue, add an appropriate amount of 1×Assay Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Plant tissues: Weigh 0.01-0.1 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℃. Use supernatant for assay, and place it on ice to be tested.
3. Cells or bacteria: Collect 1-5×10^6 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℃. Use supernatant for assay, and place it on ice to be tested.
4. Plasma and serum: Diluted 100 times or more with 1×Assay Buffer to be tested.
5. Liquid sample: Centrifuge at 10,000 g for 10 min at 4℃ 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℃. 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℃, 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℃ for 10 min, the supernatant of the acetone extract was diluted with 50% acetone. The total ORAC value was calculated by combining the results of acetone extracts from the water-soluble and insoluble fractions.

Note: The samples extracted by this kit can also be used for the determination of KTB1502.

Assay Procedure

1. Preheat the fluorescence microplate reader to 37℃. 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	25	0	0
Standard	0	25	0
Supernatant	0	0	25
1×Reagent Ⅰ	150	150	150
Mix well and incubated for 37℃ for 30 min			
Working Reagent Ⅱ	25	25	25

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℃.

Note: 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. The final measured value of the blank Well should be less than 10% of the initial value.

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.

The ORAC 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

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

RFU_0=0 min relative fluorescence value.

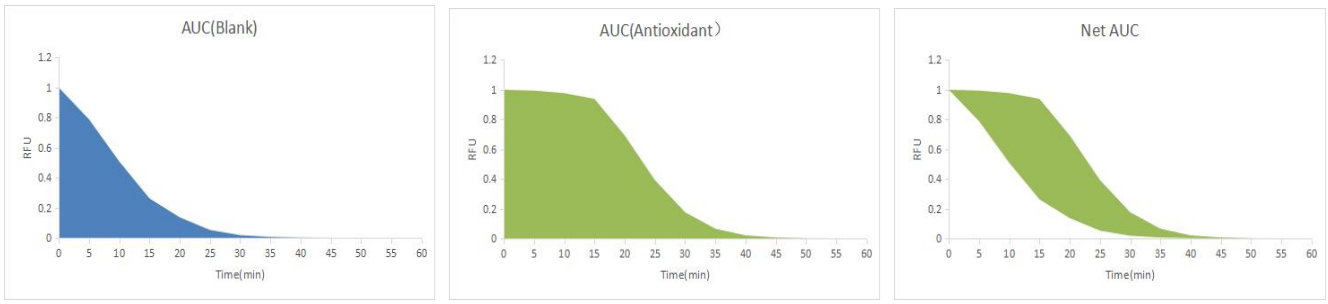
RFU_x=x min relative fluorescence value. (For example, RFU_5 is the relative fluorescence value at 5 min).

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 ORAC value in micromole per liter of Trolox equivalent (TE) or TE per gram 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

1. Typical standard curve:

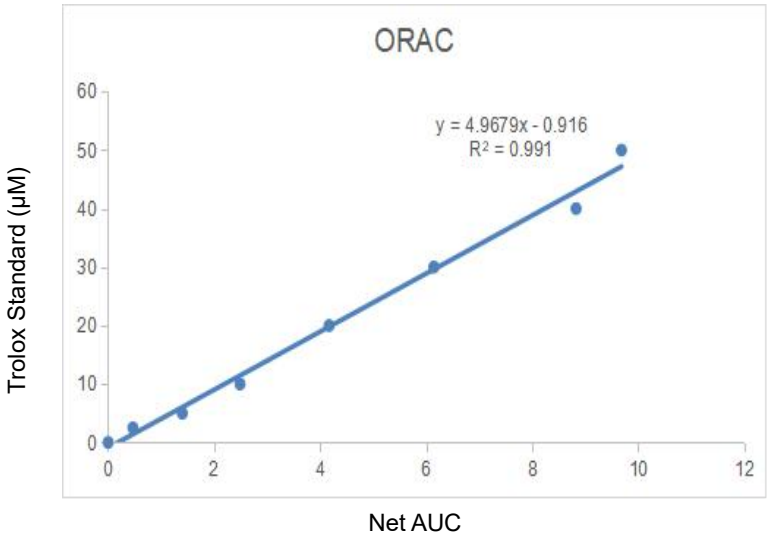


Figure 1. Standard curve for ORAC.

Examples:

Take 0.1 g animal tissue and add 1 mL 1×Assay Buffer to homogenize and grind, take the supernatant, dilute it by 50 times, then follow the measurement procedure, and measure with a black 96-well plate:

The AUC of the sample is 6.715, the AUC of the blank is 2.820, the net AUC=AUC (sample)-AUC (blank)=6.715-2.820=3.895, the standard curve is y=4.9679x-0.916, the Trolox concentration is 18.43 μM, the ORAC value (sample)=18.43 μM×50 (dilution factor)=921.5 μM TE=921.5 μmol TE/L.

Recommended Products

Catalog No.	Product Name
KTB1502	CheKine™ Hydroxyl Radical Antioxidant Capacity (HORAC) Fluorometric Assay Kit
KTB1500	CheKine™ Micro Total Antioxidant Capacity (TAC) Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity 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.