（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K807-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（505-545 nm）

Detection range：17.39–125 μmol VC/L

Elabscience® DPPH Free Radical Scavenging Capacity Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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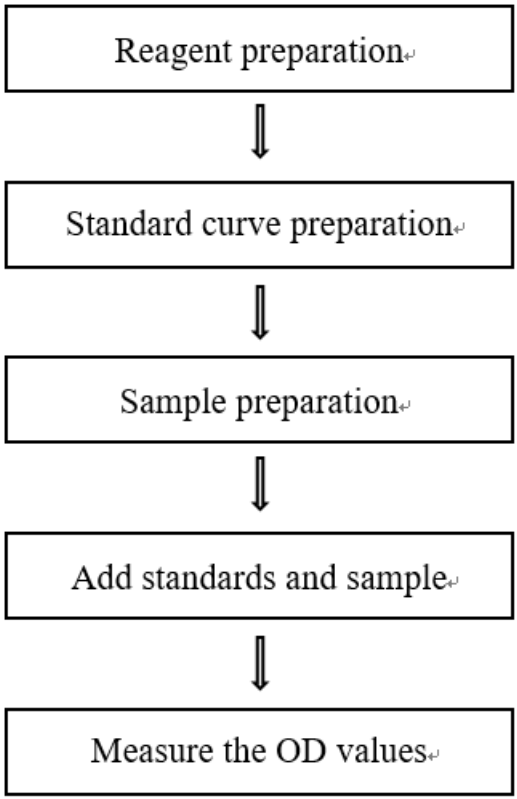
Assay summary



Intended use

This kit can be used for detection of DPPH free radical scavenging capacity in serum, plasma, tissue and cells samples.

Detection principle

DPPH is a synthetic organic free radical that can be used to evaluate the antioxidant activity of antioxidants. Its single electron has the maximum absorption at 525 nm, and the DPPH radical scavenging ability of the sample can be calculated by measuring the change of OD value before and after the addition of antioxidants.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Chromogenic Agent	Powder ×1 vial	2-8℃, 12 months, shading light
Reagent 2	Standard	Powder ×2 vials	2-8℃, 12 months, shading light
	15 mL Brown Plastic Bottle	1 bottle	
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (505-545 nm, optimum wavelength: 525 nm), Ultrasonotor

Reagents:

Anhydrous ethanol, 80% Ethanol

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of chromogenic working solution:

Dissolve one vial of chromogenic agent with 1 mL of absolute ethanol, sonicated for 1 min with ultrasonotor to mix well. Take all the obtained solution to a 15 mL brown plastic bottle, then add 11 mL anhydrous ethanol to the brown plastic bottle, and sonicated for 5 min before use (If there is no ultrasonotor, The solution can be transferred to a brown plastic bottle and stand at room temperature for 2 hours protected from light). Store at 2-8℃ for 2 weeks protected from light.

③ The preparation of 12.5 mmol/L standard solution:

Dissolve one vial of standard with 1 mL of anhydrous ethanol, mix well to dissolve. Store at 2-8℃ for 1 week protected from light.

④ The preparation of 125 μmol/L standard solution:

Add 10 μL of 12.5 mmol/L standard solution and 990 μL of anhydrous ethanol, mix well. The 125 μmol/L standard solution should be prepared on spot.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 125 μmol/L standard solution with anhydrous ethanol to a serial concentration. The recommended dilution gradient is as follows: 0, 25, 50, 62.5, 75, 87.5, 100, 125 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**25**	**50**	**62.5**	**75**	**87.5**	**100**	**125**
125 μmol/L standard (μL)	0	40	80	100	120	140	160	200
Anhydrous ethanol (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation:

Serum (plasma): detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL 80% ethanol with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL 80% ethanol with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Horse serum	1
Dog plasma	1
Mouse serum	1
Rat serum	1
10% Rat liver tissue homogenate	3-6
10% Rat heart tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Cow liver tissue homogenate	4-8
10% Mouse lung tissue homogenate	3-6
10% Mouse stomach tissue homogenate	3-6
1.92×10^6 CHO cells	1
2.164×10^6 Molt-4 cells	1

Note: The diluent is 80% ethanol. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

Chromogenic working solution should be sonicated for 5 min or stand at room temperature for 2 hours protected from light.

Operating steps

For tissue and cell samples:

① Standard well: add 80 μL of standard with different concentrations into standard wells.
Sample well: add 80 μL of sample into sample wells.
Control well: add 80 μL of sample into control wells.

② Add 100 μL of chromogenic working solution to standard wells and sample wells.

③ Add 100 μL of anhydrous ethanol to control wells.

④ Mix fully for 5 s with microplate reader and stand at room temperature for 10 min protected from light. Measure the OD value of each well at 525 nm with microplate reader.

For serum(plasma) sample:

① Standard tube: add 400 μL of standard with different concentrations into 2 mL EP tubes.
Sample tube: add 400 μL of sample into 2 mL EP tubes.
Control tube: add 400 μL of sample into 2 mL EP tubes.

② Add 500 μL of chromogenic working solution to standard tubes and sample tubes.

③ Add 500 μL of anhydrous ethanol to control tubes.

④ Mix fully and stand at room temperature for 10 min protected from light.

⑤ Centrifuge at 4000×g for 5 min at 4℃, then take 180 μL of supernatant to corresponding wells of microplate respectively. Measure the OD value of each well at 525 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

DPPH free radical scavenging capacity (μmol VC/L)

$$= (A_{blank} - (A_{sample} - A_{control}) - b) \div a \times f$$

DPPH free radical scavenging rate (%)

$$= (A_{blank} - (A_{sample} - A_{control})) \div A_{blank} \times 100\%$$

[Note]

A_{blank}: The OD value of blank well (A_{blank} is the OD value when the standard concentration is 0).

A_{sample}: The OD value of sample well.

$A_{control}$: The OD value of control well.

f: Dilution factor of the sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.055	0.080	0.110
%CV	3.0	3.8	0.8

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.055	0.080	0.110
%CV	9.5	4.5	2.1

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 97%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.055	0.08	0.11
Observed Conc. (mmol/L)	0.054	0.077	0.106
Recovery rate (%)	99	96	96

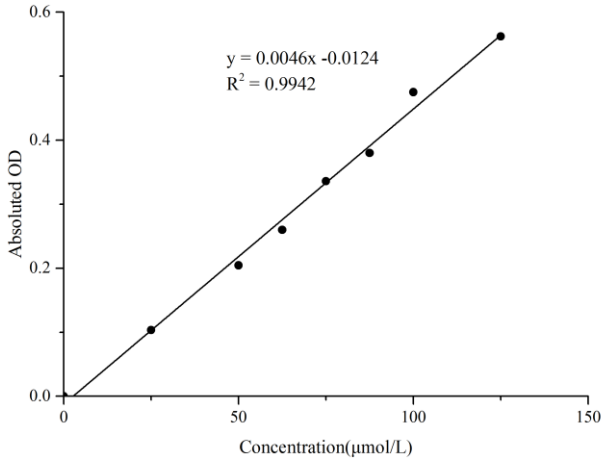
Sensitivity

The analytical sensitivity of the assay is 17.39 μmol VC/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only.

Concentration (μmol/L)	0	25	50	62.5	75	87.5	100	125
OD value	1.602	1.496	1.394	1.353	1.262	1.235	1.115	1.037
	1.608	1.507	1.407	1.337	1.276	1.215	1.145	1.049
Average OD	1.605	1.502	1.401	1.345	1.269	1.225	1.130	1.043
Absoluted OD	0	0.104	0.205	0.260	0.336	0.380	0.475	0.562



Appendix II Example Analysis

Example analysis:

Dilute 10% rat liver tissue homogenate, dilute for 4 times, then take 80 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0046 x - 0.0124, A_{blank} = 1.605, A_{sample} = 1.335, $A_{control}$ = 0.041, the calculation result is:

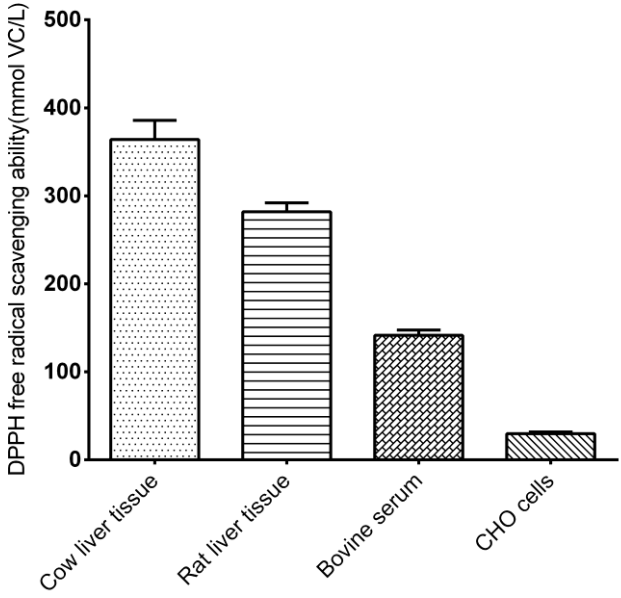
DPPH free radical scavenging capacity (μmol VC/L)

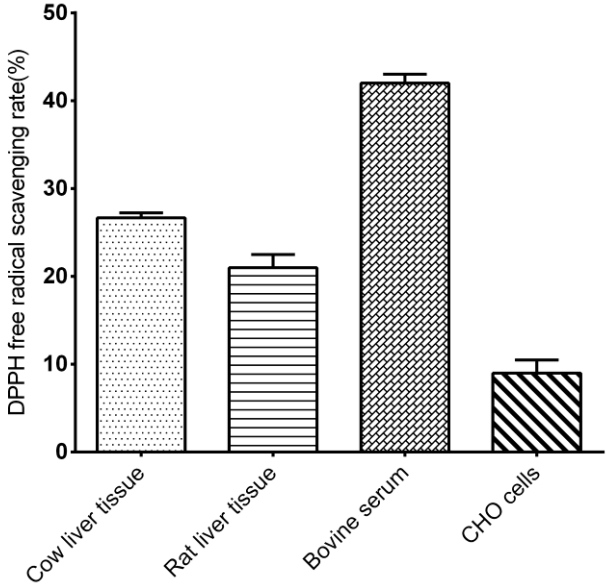
= (1.605 – (1.335 – 0.041) + 0.0124) ÷ 0.0046 × 4 = 281.22 μmol VC/L

DPPH free radical scavenging rate (%)

= (1.605 – (1.335 – 0.041)) ÷ 1.605 × 100% = 19%

Detect 10% cow liver tissue homogenate (dilute for 4 times), 10% rat liver tissue homogenate (dilute for 4 times), bovine serum and 1.92 ×10^6 CHO cells, according to the protocol, the result is as follows:




DPPH free radical scavenging rate(%)
50
40
30
20
10
0
Cow liver tissue
Rat liver tissue
Bovine serum
CHO cells

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.