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CheKine™ Micro ABTS Free Radical Scavenging Capacity Assay Kit

Cat #: KTB1093 Size: 48 T/48 S 96 T/96 S

	Micro ABTS Free Radical Scavenging Capacity Assay Kit		
REF	**Cat #:** KTB1093	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant Tissues, Red Wine, and other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

The total antioxidant level is composed of various antioxidant substances and antioxidant enzymes in the tested object. The ABTS method is the most widely used indirect detection method, suitable for measuring the antioxidant capacity of both hydrophilic and lipophilic substances. CheKine™ Micro ABTS Free Radical Scavenging Capacity Assay Kit provides a simple, convenient, and rapid method for measuring ABTS radical scavenging activity, suitable for plant tissues, red wine, and other liquid samples. The principle is that ABTS is oxidized to generate a stable blue-green cationic radical, $ABTS^+$, which is soluble in aqueous or acidic ethanol media and exhibits maximum absorption at 734 nm. After adding the test substance to the $ABTS^+$ solution, the antioxidant components in the sample react with $ABTS^+$, causing the reaction system to fade in color. The change in absorbance is measured at 734 nm, and the antioxidant capacity of the test substance is quantified by comparison with a reference system using vitamin C.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	100 mL	100 mL×2	4℃
Reagent Ⅰ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Vitamin C (Positive Control)	Powder×1 vial	Powder×1 vial	4℃, 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

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 734 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Low-temperature centrifuge, ice maker
- Deionized water, 95% ethanol
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Pre-cool before use. Store at 4℃.

Reagent Ⅰ Stock Solution: Prepare 24 h before use; for the 48 T kit, add 2.5 mL of deionized water to Reagent Ⅰ and dissolve completely, and for the 96 T kit, add 5 mL of deionized water to Reagent Ⅰ and dissolve completely. **Leave at room temperature in the dark for 24 h before use**. Unused reagents can be stored at 4℃ in the dark for up to one week.

Notes: Reagent Ⅰ is present in a small amount and is not visible to the naked eye. Please do not pour it out; once dissolved. The dissolved solution appears black-green in color.

Working Reagent Ⅰ: Prepare immediately before use; dilute the Reagent Ⅰ Stock Solution 50 times with 95% ethanol according to the required amount. Use it immediately after preparation and within 30 min.

Vitamin C Working Reagent (Positive Control): Prepare before use. Add 1 mL of deionized water and mix thoroughly to prepare a 10 mg/mL Vitamin C solution. Store at 4℃, protected from light to two weeks. This is used as a positive control.

Preparation of Positive Control: If a linear relationship is required, it is recommended to 10 times dilute the 10 mg/mL Vitamin C solution with deionized water to 1 mg/mL, then prepare working solutions of 0.1, 0.05, 0.025, 0.0125, 0.0063, 0.0031, 0.0016 mg/mL Vitamin C for use. Refer to the table below for dilutions.

Num.	Vitamin C Volume (μL)	Deionized water Volume (μL)	Concentration (mg/mL)
Std.1	50 μL of 1 mg/mL	450	0.1
Std.2	200 μL of 0.1 mg/mL	200	0.05
Std.3	200 μL of 0.05 mg/mL	200	0.025
Std.4	200 μL of 0.025 mg/mL	200	0.0125
Std.5	200 μL of 0.0125 mg/mL	200	0.0063
Std.6	200 μL of 0.0063 mg/mL	200	0.0031
Std.7	200 μL of 0.0031 mg/mL	200	0.0016

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

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.

1. Preparation of Plant Samples: Weigh out approximately 0.1 g of the sample, add 1 mL of Extraction Buffer, and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃, collect the supernatant, and keep it on ice for subsequent analysis.

2. Liquid Samples such as Red Wine, Juice, etc.: Pipette 100 μL of the sample solution into 900 μL of Extraction Buffer, mix thoroughly by vortexing. Centrifuge at 10,000 g for 10 min at 4℃, collect the supernatant, and keep it on ice for subsequent analysis.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 734 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are performed in a 96-well plate or micro glass cuvette.):

Reagent	Test Well (μL)	Blank Well (μL)	Positive Control Well (μL)
Sample Supernatant	10	0	0

Different Concentrations of Vitamin C Solution	0	0	10
Deionized water	0	10	0
Working Reagent Ⅰ	180	180	180
Mix thoroughly and incubate at room temperature in the dark for 5 min. Then, measure the absorbance at 734 nm. Record these measurements as A_{Test}, A_{Blank}, and $A_{Positive\ Control}$, respectively.			

Note: The positive control well and the blank well need to be measured only 1-2 times. The ability of different samples to scavenge ABTS radicals may vary significantly. It is recommended to conduct a preliminary experiment with 2-3 samples that are expected to show large differences before performing the full experiment.If the scavenging rate is less than 5%, the sample amount can be appropriately increased, for example, by increasing the volume of the liquid sample. If the scavenging rate exceeds 90%, the sample can be further diluted with Extraction Buffer, and the calculation result should be multiplied by the dilution factor, or the amount of sample used for extraction can be reduced.This translation maintains the technical details and context of the original instructions, ensuring clarity for experimental procedures.

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.

1. Formula for calculating the radical scavenging rate of the positive control:

ABTS Radical Scavenging Rate (D_{VC}%)**=[(A_{Blank}-$A_{Positive\ Control}$)÷A_{Blank}]×100%**

2. Formula for calculating the radical scavenging rate of the sample:

ABTS Radical Scavenging Rate (D%)**=[(A_{Blank}-A_{Test})]÷A_{Blank}]×100%**

Precautions

1. Try to avoid using samples that turn blue or nearly blue under alkaline conditions, as they may interfere with the detection results of this kit.
2. Detergents such as Tween, Triton, and NP-40, as well as reducing agents like DTT and β-mercaptoethanol, which affect redox reactions, should not be added to the samples.
3. The ability of different samples to scavenge ABTS radicals may vary significantly. If comparing the ABTS radical scavenging capacity of different samples, it is recommended to test equal amounts of the same batch of samples.
4. It is recommended to extract and test the samples on the same day.

Typical Data

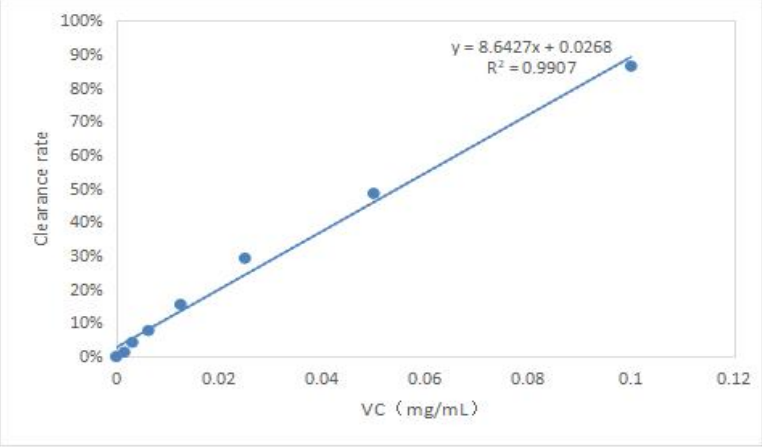

Figure 1. Positive control curves

Recommended Products

Catalog No.	Product Name
KTB4010	CheKine™ Micro Soil Nitrate Reductase (S-NR) Activity Assay Kit
KTB4021	CheKine™ Micro Leucine Arylamidase (LAP) 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.