CheKine™ Micro Betaine Content Assay Kit

Cat #: KTB2350 Size: 48 T/96 T

	Micro Betaine Content Activity Assay Kit		
REF	**Cat #:** KTB2350	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Betaine is a kind of quaternary ammonium type water-soluble alkaloid widely distributed in animals, plants and microorganisms. It is the oxidation product of choline in organism. It can enhance immunity, reduce blood lipid, resist oxidation and anti-tumor. It can also be used as a methyl donor to promote protein and fat metabolism, increase appetite, relieve stress, regulate osmotic pressure, stabilize vitamins and other biological functions It is widely used in chemical industry, medicine, food additive and other fields. CheKine™ Micro Betaine Content Assay Kit can be used to detect biological samples such as animal and plant tissues. In the kit, under strong acid conditions, betaine reacts with Raynaud salt to produce precipitation. The precipitation is dissolved in acetone to form a red solution. There is a characteristic absorption peak at 525 nm. The absorption value at 525 nm is determined to obtain the content of betaine in the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	1×2	1×3	4℃, protected from light
Standard	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 525 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge
- Deionized water, ether, HCl, acetone
- Homogenizer or mortar, 40-mesh sieve

Reagent Preparation

Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent Ⅰ: Prepared before use. Add 15 mL of distilled water to each bottle, adjust the pH to 1 with concentrated HCl, stir for 30 min. After filtration, making up to 20 mL with distilled water.

Reagent Ⅱ: 99% ether, take ether 59.4 mL, add deionized water 0.6 mL, mix well. **(Required but not provided)**

Reagent Ⅲ: 70% acetone, take acetone 42 mL, add deionized water 18 mL, mix well. **(Required but not provided)**

Standard: Prepared before use.Add 1 mL deionized water to fully dissolve the standard to betaine solution of 10 mg/mL. Store at 4℃ for 6 months, protected from light.

Standard preparation: Use the 10 mg/mL betaine standard solution and further dilute it to the standard as shown in the following table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (mg/mL)
Std.1	45 μL 10 mg/mL Standard	455	0.9
Std.2	40 μL 10 mg/mL Standard	460	0.8
Std.3	35 μL 10 mg/mL Standard	465	0.7
Std.4	30 μL 10 mg/mL Standard	470	0.6
Std.5	25 μL 10 mg/mL Standard	475	0.5
Std.6	20 μL 10 mg/mL Standard	480	0.4
Std.7	15 μL 10 mg/mL Standard	485	0.3
Std.8	10 μL 10 mg/mL Standard	490	0.2

Notes: Always prepare fresh Standards per use; Diluted Std. 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.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

Tissues: Weigh 0.02 g dried tissue that has passed 40-mesh sieve, add 0.8 mL deionized water, extract at 60℃ for 30 min and fluctuated continuously during the period. Add 200 μL Extraction Buffer, mix it well. Centrifuge at 10,000 g for 10 min at 25℃, and take the supernatant to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 525 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following operations are operated in 1.5 mL EP tube)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	300
Standard	0	300	0
Deionized water	300	0	0
Reagent Ⅰ	300	300	300
Mix well, react at 4℃ for 2 h, centrifuge at 14,000 g for 10 min at 25℃, discard the supernatant.			
Reagent Ⅱ	500	500	500

Mix well, centrifuge at 14,000 g for 10 min at 25℃, discard the supernatant. Put it in the ventilator to make the residual ether volatilize naturally.			
Reagent III	300	300	300

3. The shock causes the precipitation to be fully dissolved, add 200 μL in the 96-well plate or microglass cuvette, detect the absorbance at 525 nm. The Blank Well is recorded as A_{Blank}, the standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

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. If ΔA_{Test} is less than 0.001, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation y=kx+b. The determination of ΔA_{Test} is brought into the equation to get x (mg/mL).

2. Calculation of the betaine content

Calculated by dry weight of samples

Betaine(mg/g dry weight)=$x\times V_{Standard}\div(V_{sample}\div V_{Total\ sample}\times W)$**=x÷W**

$V_{Standard::}$ The volume of the standard, 0.3 mL; $V_{sample:}$ The volume of the sample in the reaction, 0.3 mL; $V_{Total\ sample}$: The volume of Extraction Buffer, 1 mL; W: Sample weight, g

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

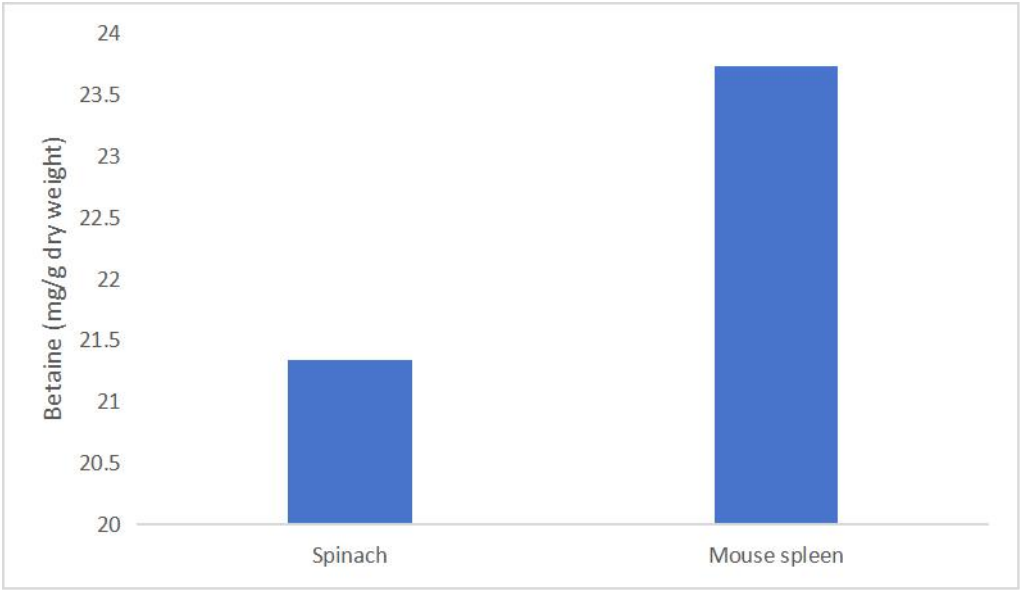

Figure 1. Determination of betaine content in spinach and mouse spleen by this kit.

Recommended Products

Catalog No.	Product Name

KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Activity Assay Kit
KTB1110	CheKine™ Lactate Dehydrogenase (LDH) Activity Assay Kit

Disclaimer

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