CheKine™ Micro Saponin Content Assay Kit

Cat #: KTB4055 Size: 48 T/48 S 96 T/96 S

	Micro Saponin Content Assay Kit		
REF	**Cat #:** KTB4055	LOT	**Lot #:** Refer to product label
	Detection range: 18-1500 μg/g (Taking ginsenoside Re as Standard) 9-450 μg/g (Taking oleanolic acid as Standard)		**Sensitivity:** 18 μg/g (Taking ginsenoside Re as Standard) 9 μg/g (Taking oleanolic acid as Standard)
	Applicable sample: Plant Tissue		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Saponin is a kind of natural substance widely existing in plant seeds, stems, roots and other parts, and its aglycone is triterpenoids or spirostanes. Many Chinese herbal medicines, such as ginseng, polygala tenuifolia, platycodon grandiflorum, licorice, Anemarrhena asphodeloides and Bupleurum chinense, contain saponin, which has many biological activities and application values, such as anti-inflammatory, antibacterial, hypolipidemic, hypoglycemic and antioxidant. CheKine™ Micro Saponin Content Assay Kit can detect biological samples such as plant tissue. In this kit, the total saponin in the sample were extracted by ultrasonic wave, and the color reaction between saponins and vanillin occurred under acidic conditions. The product had a characteristic absorption peak at 589 nm, and the content of total saponins could be quantitatively detected by the change of absorption value.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃, protected from light
Reagent Ⅰ	15 mL	30 mL	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 589 nm
- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tube
- Water bath, centrifuge, ultrasonic wave cleaner, 100 mesh sieve
- Deionized water, perchloric acid, acetic acid
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light. Reagent Ⅰ will crystallize at 4℃, which is normal. In case of crystallization, it can be heated in a 30℃ water bath to help dissolve, and the reagent must be completely dissolved before use.

Note: Reagent Ⅰ has certain irritation, so personal protection is recommended during use.

Sample Preparation

Note: Note: It is recommended to use fresh soil samples.

Plant Tissue: The sample is dried, crushed and sieved by a 100 mesh sieve. Weigh 0.05 g dried tissue, add 1 mL Extraction Buffer . Cover tightly and ultrasonic break 1 h. Centrifuge at 8,000 g for 10 min at 25℃. Use supernatant for assay.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 589 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Test Tube（μL）	Blank Tube（μL）
Sample	500	0
Extraction Buffer	0	500
Open the lid and evaporate to dryness at 70℃.		
Reagent Ⅰ	200	200
Perchloric acid	800	800
Mix well and incubate at 55℃ for 20 min. Centrifuge at 8,000 g for 10 min at 25℃. Use supernatant for assay. The following operations are operated in the 96-well microplate or microglass cuvette:		
Supernatant	40	40
Acetic acid	200	200

Mix well, record the absorbance value at 589 nm. The Blank Well is recorded as A_{Blank}, the Test Well is marked as A_{Test}. Finally calculate $\Delta A=A_{Test}-A_{Blank}$.

Note: The Blank Well only need to be done once or twice. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.01, increase the sample quantity appropriately. If ΔA is larger than 1.5, 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 ($A_{Standard}$-A_{Blank}) as the y-axis, draw the standard curve and obtain the standard equation. The determination of ΔA is brought into the equation to get x (μg/mL).

Calculate by taking ginsenoside Re as standard y=0.001x+0.0045, R^2=0.9971.

Calculate by taking oleanolic acid as standard, y=0.0035x-0.0214, R^2=0.9972.

2. Calculation of the saponin content

Saponin content (μg/g dry weight)=(x×V_{Sample})÷(V_{Sample}÷V_{Total}×W)**=x÷W**

V_{Sample}: add sample volume, 0.5 mL; V_{Total}: add Extraction Buffer volume to sample, 1 mL; W: dry weight of sample, g.

Typical Data

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

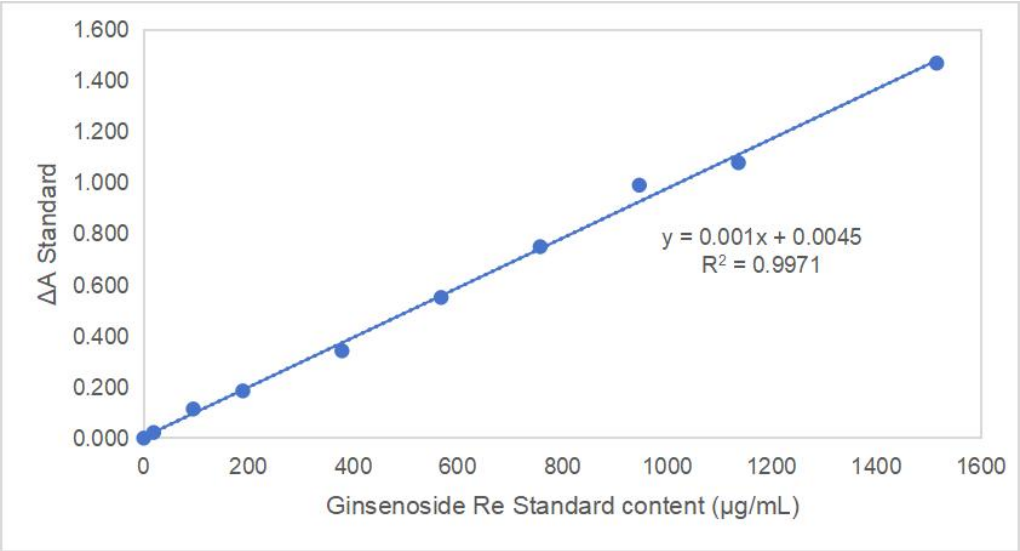

Figure 1. Standard curve of ginsenoside Re.

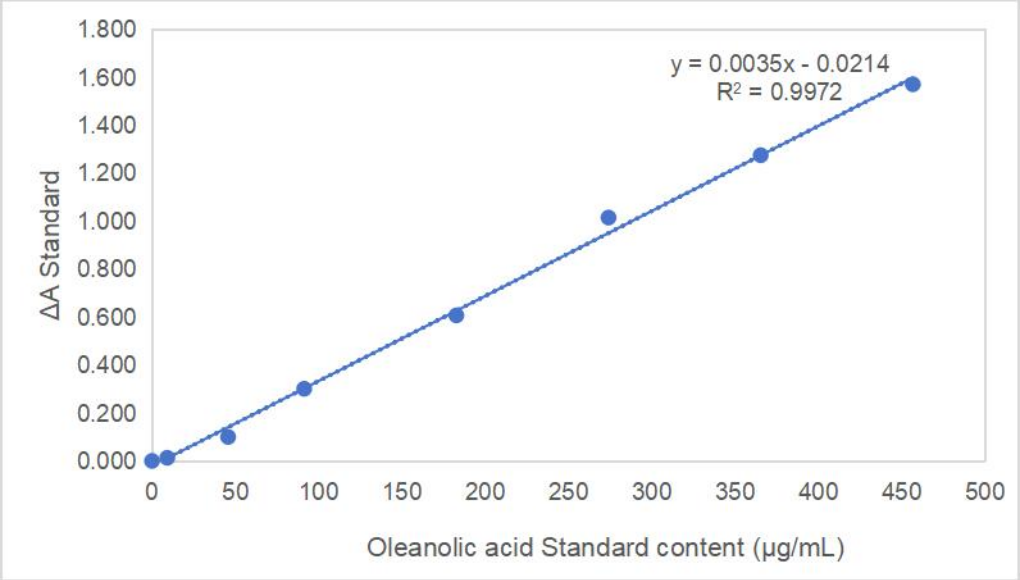

Figure 2. Standard curve of oleanolic acid.

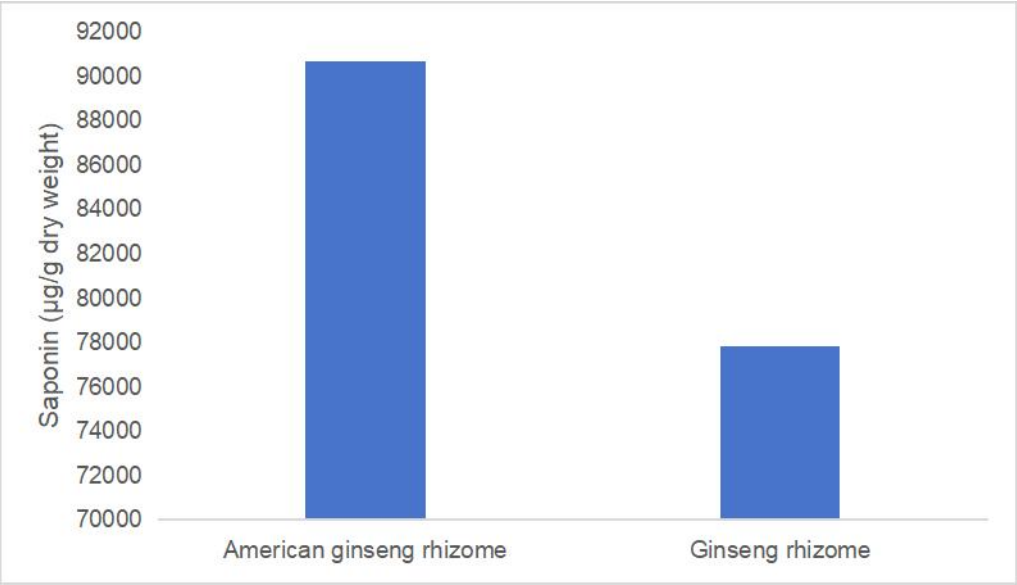

Figure 2. Determination saponin content in American ginseng rhizome and ginseng rhizome sample by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Content 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.