CheKine™ Micro Soli Glutaminase (S-GLS) Activity Assay Kit

Cat #: KTB4031 Size: 48 T/48 S 96 T/96 S

	Micro Soli Glutaminase (S-GLS) Activity Assay Kit		
REF	**Cat #:** KTB4031	LOT	**Lot #:** Refer to product label
	Applicable sample: Soli		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Soli glutaminase (S-GLS), which is mainly found in higher animals, some bacteria and plant roots, catalyzes the hydrolysis of glutamine to glutamate and ammonia. It plays an important role in the regulation of nitrogen metabolism, especially the content of free ammonia and urea metabolism. CheKine™ Micro Soli Glutaminase (S-GLS) Activity Assay Kit can detect biological samples such as soli. In this kit, S-GLS catalyzes the hydrolysis of glutamine to L-glutamate and ammonia, and its enzymatic activity can be calculated by detecting the rate of ammonia increase using Nessler's reagent.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	12 mL	24 mL	4℃
Reagent Ⅱ	Powder×2 vials	Powder×2 vials	4℃, protected from light
Reagent Ⅲ	70 mL	70 mL×2	4℃, protected from light
Reagent Ⅳ	4 mL	8 mL	4℃
Reagent Ⅴ	2.5 mL	5 mL	4℃
Reagent Ⅵ	2.5 mL	5 mL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃

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 420 nm
- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tube
- Water bath, freezing centrifuge, 30-50 mesh sieve
- Deionized water, toluene

Reagent Preparation

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent II: Prepared before use. Add 25 mL deionized water to each Reagent II for 48 T, and 50 mL deionized water to each Reagent II for 96 T to fully dissolve. Working Reagent II is freshly prepared.

Reagent III: Ready to use as supplied. Store at 4℃, protected from light.

Reagent IV: Ready to use as supplied. Store at 4℃.

Reagent V: Ready to use as supplied. Store at 4℃.

Reagent VI: Ready to use as supplied. Store at 4℃, protected from light.

Note: Reagent III has an irritating odor, Reagent IV is corrosive, and Reagent VI is toxic, so it is recommended to experiment in a fume hood.

Standard: Prepared before use. Add 1 mL deionized water to a bottle, dissolve thoroughly, that is 100 μmol/mL ammonia Standard. The remaining reagent can be stored at 4℃ for 1 month.

0.3125 μmol/mL Standard: Prepare 0.3125 μmol/mL Standard by diluting 10 μL 100 μmol/mL ammonia Standard into 3,190 μL deionized water. Using 0.3125 μmol/mL Standard for subsequent detection.

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

Sample Preparation

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

Fresh soil samples naturally air dried or air dried in an oven at 37℃ and sieved through 30-50 mesh sieve.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 420 nm, visible spectrophotometer was returned to zero with deionized water.

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

Reagent	Test Tube	Control Tube	Standard Tube	Blank Tube
Sample (g)	0.1	0.1	0	0
Toluene (μL)	25	25	0	0
Shake and mix well, and let stand at room temperature for 15 min.			0	0
Reagent I (μL)	100	100	0	0
Working Reagent II (μL)	400	0	0	0
Deionized Water (μL)	0	400	0	0
Mix thoroughly, put it in an incubator at 37℃ to react for 2 h.			0	0
Reagent III (μL)	525	525	0	0
Mix thoroughly, centrifuge at 8,000 g for 10 min at 25℃, take the supernatant. The following operations are operated in 96-well microplate or microglass cuvette **in turn**:			0	0
Supernatant (μL)	130	130	0	0
0.3125 μmol/mL Standard (μL)	0	0	130	0
Deionized Water (μL)	0	0	0	130

Reagent Ⅳ (μL)	30	30	30	30
Reagent Ⅴ (μL)	20	20	20	20
Reagent Ⅵ (μL)	20	20	20	20

Mix and shaking for 5 s, **immediately** record the absorbance value at 420 nm. The Blank Tube is recorded as A_{Blank}, the Standard Tube is marked as $A_{Standard}$, the Test Tube is marked as A_{Test}, and the Control Tube is marked as $A_{Control}$. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: (1) Each test tube needs to be equipped with a control tube, standard tube and blank tube only need to be done once or twice. (2) 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 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.8, decrease the sample quantity appropriately. (3) After adding Reagent Ⅵ, it is necessary to measure the absorption value immediately after mixing and shaking for 5 s. In order to avoid experimental errors, it is suggested that no more than 3 sample wells should be detected each time.

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.

Calculation of S-GLS activity:

Active unit definition: Catalysis of glutamine to 1 nmol ammonia per minute per gram of sample was defined as one unit of enzyme activity at 37℃.

$S\text{-}GLS(U/g\ soli)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total}\div W\div T\times 1{,}000$**$=2.734\times\Delta A_{Test}\div\Delta A_{Standard}\div W$**

$C_{Standard}$: Standard concentration, 0.3125 μmol/mL; V_{Total}: total reaction volume, 1.05 mL; T: reaction time, 2 h=120 min; W: weight of sample, g; 1000: conversion factor, 1 μmol=1,000 nmol.

Typical Data

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

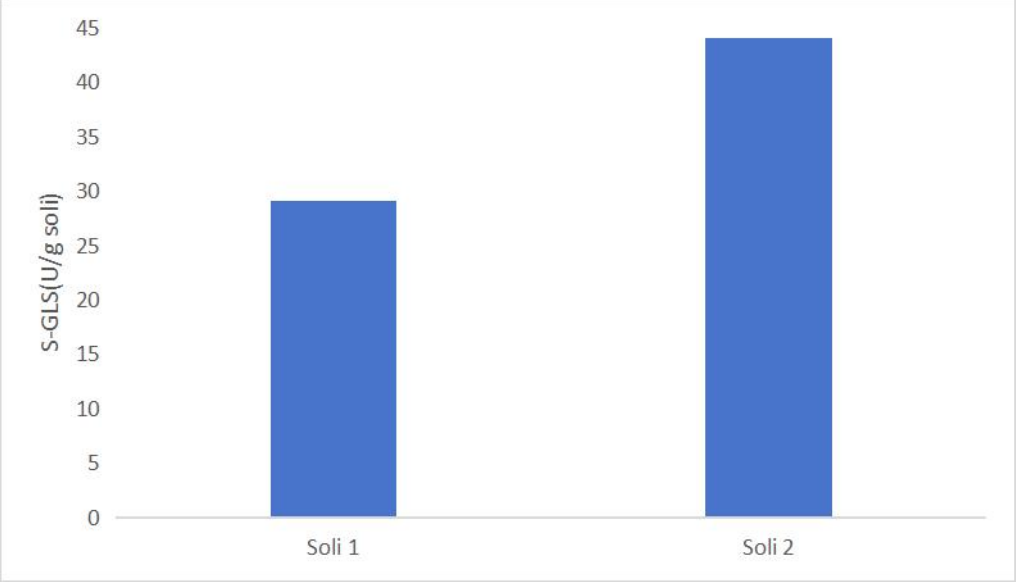

Figure 1. Determination S-GLS activity in soli sample by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB4012	CheKine™ Micro Soil Nitrate Nitrogen Assay Kit
KTB4014	CheKine™ Micro Acid Soil Available Phosphorous Assay Kit
KTB4041	CheKine™ Micro Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit
KTB4050	CheKine™ Micro Soil Catalase (S-CAT) 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.