CheKine™ Micro Soli Neutral Protease (S-NPT) Activity Assay Kit

Cat #: KTB4034 Size: 48 T/48 S 96 T/96 S

	Micro Soli Neutral Protease (S-NPT) Activity Assay Kit		
REF	**Cat #:** KTB4034	LOT	**Lot #:** Refer to product label
	Applicable samples: Soli		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Soil protease is involved in the transformation of amino acids, proteins and other organic compounds containing protein nitrogen in soil. Its hydrolysate is one of the nitrogen sources of higher plants. Soil neutral protease catalyzes protein hydrolysis in neutral environment, which is related to soil organic matter content, nitrogen and other soil properties. CheKine™ Micro Soli Neutral Protease (S-NPT) Activity Assay Kit can be used to detect biological samples such as soli. In the kit, under neutral conditions, casein can be hydrolyzed by soil neutral protease to produce tyrosine. In alkaline condition, tyrosine reduced phosphomolybdic acid compound to form tungsten blue, which has an absorbance peak at 680 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	20 mL	40 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅲ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅳ	Powder×1 vial	Powder×1 vial	4℃
Reagent Ⅴ	7.5 mL	15 mL	4℃, protected from light
Reagent Ⅵ	2.5 mL	5 mL	4℃
Standard	1 mL	1 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 680 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, centrifuge, 30-50 mesh sieve

• Deionized water

Reagent Preparation

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

Working Reagent II: Prepared before use. 48 T add 6 mL deionized water, 96 T add 12 mL deionized water to fully dissolve; The remaining reagent can also be stored at 4℃, protected from light for 1 month.

Working Reagent III: Prepared before use. 48 T add 2 mL Reagent Ⅵ, 96 T add 4 mL Reagent Ⅵ, and dissolve by magnetic stirring in boiling water bath. (You can cover the beaker with a layer of fresh-keeping film, pay attention to observation, avoid all evaporation of water, generally heat for 15-30 min, the reagent is supersaturated, and the use of insoluble particles will not be affected after full mixing.) Then 48 T add 8 mL Reagent Ⅰ, 96 T add 16 mL Reagent Ⅰ, fully mixed for use. The remaining reagent can also be stored at 4℃, protected from light for 1 month.

Working Reagent IV: Prepared before use. 48 T add 35 mL deionized water, 96 T add 70 mL deionized water to fully dissolve; The remaining reagent can also be stored at 4℃ for 1 month.

Reagent V: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Note: Reagent II has a pungent odor, Reagent V is toxic, so it is recommended to experiment in a fume hood.

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

Stardard： Ready to use as supplied; 20 μmol/mL Tyrosine. Equilibrate to room temperature before use; Store at 4℃, protected from light.

0.4 μmol/mL Standard: Prepared before use, take 20 μL Standard, add 980 μL deionized water, mix thoroughly, and obtain 0.4 μmol/mL Standard.

Note: 0.4 μmol/mL Standard needs to be prepared for each experiment, and the diluted 0.4 μmol/mL Standard should be used up 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 680 nm. Visible spectrophotometer was returned to zero with deionized water.

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

Reagent	Test Tube	Control Tube	Standard Tube	Blank Tube
Sample (g)	0.04	0.04	0	0
Reagent Ⅰ (μL)	0	150	0	0
Working Reagent Ⅲ (μL)	150	0	0	0
Mix thoroughly, put it in an incubator at 37℃ to react for 24 h, during which it oscillates for 5-6 times to make the soil sample fully contact with the reaction solution.			0	0
Working Reagent Ⅱ (μL)	50	50	0	0
Mix thoroughly, centrifuge at 8,000 g for 10 min at 25℃, take the supernatant. The following operations are operated in the new 1.5 mL EP tube:			0	0
Supernatant (μL)	60	60	0	0
0.4 μmol/mL Standard (μL)	0	0	60	0

Deionized Water (μL)	0	0	0	60
Working Reagent Ⅳ (μL)	280	280	280	280
Reagent Ⅴ (μL)	60	60	60	60

3. Mix thoroughly, put in 40℃ for 20 min, centrifuge at 8,000 g for 10 min at 25℃. Add 200 μL to micro glass cuvette/96-well plate, detect the absorbance at 680 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) The Blank Tube and the Standard Tube 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. (2) If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.8, supernatant after adding Working Reagent Ⅱ 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.

Calculation of the S-NPT activity

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol tyrosine in the reaction system every g sample everyday.

$S\text{-}NPT(U/g\ soil)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total\ volume}\div W\div T$**$=0.08\times\Delta A_{Test}\div\Delta A_{Standard}\div W$**

$C_{Standard}$: 0.4 μmol/mL tyrosine solution; $V_{Total\ volume}$: Reaction total volume, 0.2 mL; W: Sample weight, g; T: The reaction time, 24 h=1 d.

Typical Data

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

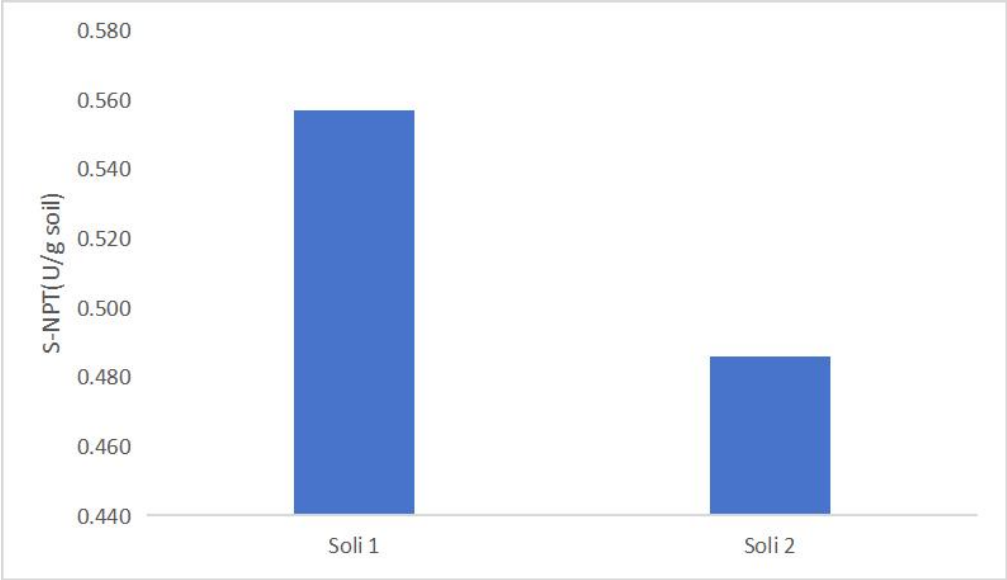

Figure 1. Determination of S-NPT activity in soli sample by this kit.

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.