CheKine™ Micro Asparagine Synthetase (AS) Activity Assay Kit

Cat #: KTB3046 Size: 48 T/96 T

	Micro Asparagine Synthetase (AS) Activity Assay Kit		
REF	**Cat #:** KTB3046	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant tissues, Cells or Bacteria, Plasma, Serum		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Asparagine synthetase (AS) is a widely distributed enzyme in living organisms belonging to the class of amino transferases. It catalyzes the transfer of an amine group from glutamine to aspartic acid. When a plant is subjected to ammonia toxicity, the formation of asparagine serves as a detoxification mechanism. CheKine™ Micro Asparagine Synthetase (AS) Activity Assay Kit is designed to quantify asparagine synthetase activity in animal and plant tissues, bacterial and cellular samples, as well as in serum (plasma). The assay principle relies on the AS enzyme's ability to catalyze the hydrolysis of L-asparagine into L-aspartic acid and ammonia. By employing Nessler's reagent to detect the rate of ammonia accumulation, the enzymatic activity of AS can be determined.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	60 mL	60 mL×2	4℃
Reagent Ⅱ	1	1×2	4℃, protected from light
Reagent Ⅲ	30 mL	60 mL	RT, protected from light
Reagent Ⅳ	2.5 mL	5 mL	RT
Reagent Ⅴ	1.5 mL	3 mL	RT
Reagent Ⅵ	1.5 mL	3 mL	RT, protected from light
Standard	1 mL	1 mL	4℃

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 420 nm
- 96-well microplate or microquartz cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tube

- Water bath, freezing centrifuge
- Deionized water
- Mortar or homogenizer (for tissue samples)

Reagent Preparation

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

Reagent II: Prepared before use. Add 25 mL deionized water to a bottle, dissolve thoroughly. Store at 4℃. This reagent is freshly prepared. The prepared Reagent II should be used up on the same day.

Reagent III: Ready to use as supplied. Store at room temperature protected from light.

Reagent IV: Ready to use as supplied. Store at room temperature.

Reagent V: Ready to use as supplied. Store at room temperature.

Reagent VI: Ready to use as supplied. Store at room temperature protected from light.

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

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.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Reagent I and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the EP tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Reagent I to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma, Serum: Test directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 420 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are operated in the 1.5 mL centrifugal tube):

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	25
Standard	0	25	0
Deionized Water	25	0	0
Reagent I	100	100	100
Reagent II	400	400	400
Mix well and incubate at 37℃ for 1 h.			
Reagent III	525	525	525
Mix well, centrifugation 8,000 g for 10 min at 25℃. The supernatant was removed and the following procedures were performed in a 96-well plate or microglass cuvette:			
Supernatant	130	130	130

Reagent Ⅳ	30	30	30
Reagent Ⅴ	20	20	20
Reagent Ⅵ	20	20	20

4.Mix thoroughly, quiescence at room temperature for 15 min, and measure the absorbance value at 420 nm. The Blank Well is recorded as A_{Blank}, the standard Well is marked as $A_{Standard}$, and 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: Each test well needs to be equipped with a control well, standard curve and 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_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.6, the sample can be appropriately diluted with Reagent Ⅰ, 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 AS activity:

1. Calculated by protein concentration

Active unit definition: Catalysis of asparagine to 1 nmol ammonia per minute per milligram of protein was defined as one unit of enzyme activity.

$AS(U/mg\ prot)=\Delta A_{Test}\div(\Delta A_{Standard}\div C_{Standard})\times V_{Total}\div(V_{Sample}\times Cpr)\div T\times 1{,}000$**$=875\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr$**

2. Calculated by sample fresh weight

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

$AS(U/g\ fresh\ weight)=\Delta A_{Test}\div(\Delta A_{Standard}\div C_{Standard})\times V_{Total}\div(W\times V_{Sample}\div V_{Total\ sample})\div T\times 1{,}000$**$=875\times\Delta A_{Test}\div\Delta A_{Standard}\div W$**

3. Calculated by number of cells or bacteria

Active unit definition: Catalysis of asparagine to 1 nmol ammonia per minute per 10^4 cells or bacteria was defined as one unit of enzyme activity.

$AS(U/10^4)=\Delta A_{Test}\div(\Delta A_{Standard}\div C_{Standard})\times V_{Total}\div(n\times V_{Sample}\div V_{Total\ sample})\div T\times 1{,}000$**$=875\times\Delta A_{Test}\div\Delta A_{Standard}\div n$**

4. Calculated by volume of liquid sample

Active unit definition: Catalysis of asparagine to 1 nmol ammonia per minute per milliliter of liquid was defined as one unit of enzyme activity.

$AS(U/mL)=\Delta A_{Test}\div(\Delta A_{Standard}\div C_{Standard})\times V_{Total}\div V_{Sample}\div T\times 1{,}000$**$=875\times\Delta A_{Test}\div\Delta A_{Standard}$**

$C_{Standard}$: the concentration of the standard, 1.25 μmol/mL; V_{Total}: total reaction volume, 1.05 mL; $V_{Sample:}$ sample volume added, 0.025 mL; $V_{Total\ sample}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; T: reaction time, 60 min. N: The number of bacteria or cells, in tens of thousands; 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.

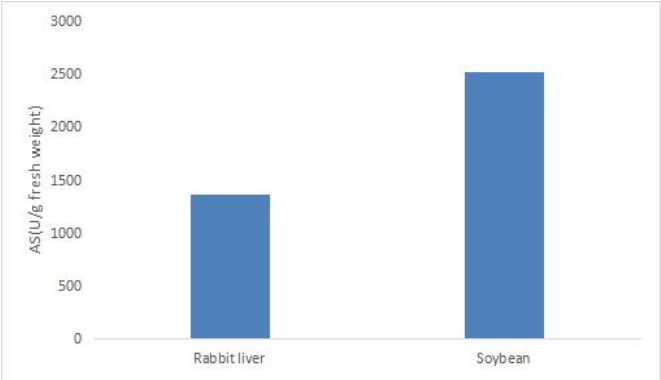

Figure 1. Determination AS activity in Rabbit liver and Soybean by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB4010	CheKine™ Micro Soil Nitrate Reductase (S-NR) Activity Assay Kit
KTB3041	CheKine™ Micro Glutamic Acid Dehydrogenase（GDH）Assay Kit
KTB3050	CheKine™ Water and Soil Nitrite Content Assay Kit
KTB3051	CheKine™ Micro Food Nitrite Assay Kit

Disclaimer

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