CheKine™ Micro N-Acetyl-β-D-Glucosidase (NAG) Activity Assay Kit

Cat #: KTB1326 Size: 48 T/96 T

	Micro N-Acetyl-β-D-Glucosidase (NAG) Activity Assay Kit		
REF	**Cat #:** KTB1326	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Bacteria or Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

N-acetyl-β-D-glucosidase (NAG) is widely distributed in various tissues. It is an intracellular lysosomal enzyme. The activity of NAG can be used for the early diagnosis of tubulointerstitial nephritis, urinary tract infection, diabetic nephropathy syndrome, hypertensive nephropathy, rejection after kidney transplantation and nephrotic syndrome. CheKine™ Micro N-acetyl-β-D-glucosidase (NAG) Activity Assay Kit can be used to detect biological samples such as animal and plant tissues, bacteria or cells, serum or plasma. In the kit, NAG decomposes N-β-acetylglucosamine to produce p-nitrophenol. It has a maximum absorption peak at 400 nm. NAG activity was calculated by measuring the change of absorbance at 400 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	1	1	-20℃, protected from light
Reagent Ⅱ	2 mL	4 mL	4℃
Reagent Ⅲ	6.5 mL	13 mL	4℃
Standard	1 mL	1 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 400 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Not for further distribution without written consent.Copyright © Abbkine, Inc.

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

Reagent Ⅰ: Prepared before use. 48 T add 1.25 mL deionized water, 96 T add 2.5 mL deionized water, fully dissolve. Inexhaustible reagents stored at -20℃ for 6 months, protected from light.

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

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

Standard： Ready to use as supplied; Liquid 1 mL×1, 5 μmol/mL p-nitrophenol solution, store at 4℃. Before use, dilute the standard 8 times with distilled water to obtain 0.625 μmol/mL standard solution.

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.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 15,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Bacteria or Cells: Collect $5×10^6$ bacteria or cells into the centrifuge tube, wash bacteria or cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 30 times (power 20% or 200 W, ultrasonic 3 s, interval 7 s). Centrifuge at 15,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma, Serum or other Liquid samples: Direct detection.

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 400 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Sample	0	0	10	10
Standard	0	10	0	0
Deionized water	45	0	0	35
Reagent Ⅰ	25	25	25	25
Reagent Ⅱ	0	35	35	0
Quickly mix well and react with 30 min at 37℃				
Reagent Ⅲ	130	130	130	130

3. Mix thoroughly, detect the absorbance at 400 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}, and the Control Well is marked as $A_{Control}$. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

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 2, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Abbkine, Inc • USA • ***www.abbkine.com***

Not for further distribution without written consent.Copyright © Abbkine, Inc.

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 NAG activity

(1) Calculated by sample protein concentration

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the generation of 1 nmol p-nitrophenol per min every mg tissue protein in the reaction system.

NAG (U/mg prot)=$\Delta A_{Test} \div (\Delta A_{Standard} \div C_{Standard}) \times 1{,}000 \times V_{Sample} \div (Cpr \times V_{Sample}) \div T$=$\mathbf{20.83 \times \Delta A_{Test} \div \Delta A_{Standard} \div Cpr}$

(2) Calculated by fresh weight of samples

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the generation of 1 nmol p-nitrophenol per min every gram tissue weight in the reaction system.

NAG (U/g fresh weight)=$\Delta A_{Test} \div (\Delta A_{Standard} \div C_{Standard}) \times 1{,}000 \times V_{Sample} \div (V_{Sample} \div V_{Total\ sample} \times W) \div T$=$\mathbf{20.83 \times \Delta A_{Test} \div \Delta A_{Standard} \div W}$

(3) Calculated by bacteria or cells

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the generation of 1 nmol p-nitrophenol per min every 10^4 bacteria or cells in the reaction system.

NAG (U/10^4)=$\Delta A_{Test} \div (\Delta A_{Standard} \div C_{Standard}) \times 1{,}000 \times V_{Sample} \div (n \times V_{Sample} \div V_{Total\ sample}) \div T$=$\mathbf{20.83 \times \Delta A_{Test} \div \Delta A_{Standard} \div n}$

(4) Calculated by volume of liquid samples

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the generation of 1 nmol p-nitrophenol per min every mL serum in the reaction system.

NAG (U/mL)=$\Delta A_{Test} \div (\Delta A_{Standard} \div C_{Standard}) \times 1{,}000 \times V_{Sample} \div V_{Total\ sample} \div T$=$\mathbf{20.83 \times \Delta A_{Test} \div \Delta A_{Standard}}$

$C_{Standard}$: Concentration of standard solution, 0.625 μmol/mL; $V_{Total\ sample}$: The volume of Extraction Buffer, 1 mL; V_{Sample}: Added sample volume to the reaction system, 0.01mL; Cpr: Sample protein concentration, mg/mL; T: Reaction time, 20 min; n: Number of bacteria or cells, ten thousand; W: Sample weight, g; 1,000: Conversion coefficient, 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.

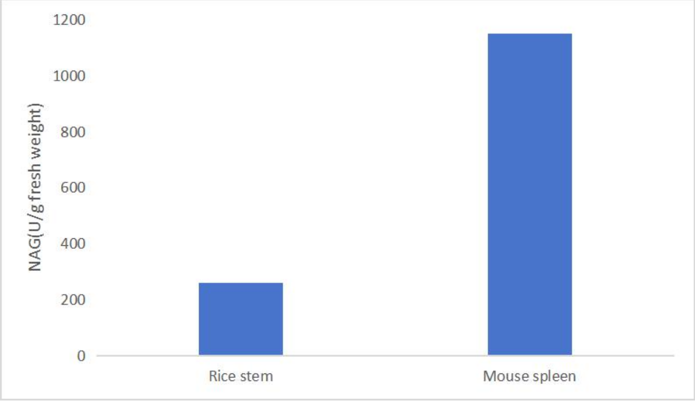

Figure 1. Determination of NAG activity in rice stem and mouse spleen by this kit.

Recommended Products

Catalog No.	Product Name

Abbkine, Inc • USA • ***www.abbkine.com***

Not for further distribution without written consent.Copyright © Abbkine, Inc.

KTB1341	CheKine™ Micro Glycogen synthase (GCS) Activity Assay Kit
KTB1380	CheKine™ Micro β-Amylase Activity Assay Kit
KTB1390	CheKine™ Micro Starch Branching Enzyme (SBE) Activity Assay Kit

Disclaimer

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

Not for further distribution without written consent.Copyright © Abbkine, Inc.