TECHNICAL MANUAL

beta-N-Acetylglucosaminidase (NAG) Activity Assay Kit

- **SKU CODE:** MAES0279
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate
- Add chromogenic agent
- Measure absorbance

2. Intended use

This kit can measure β-N-Acetylglucosaminidase (NAG) activity in serum, plasma, animal tissue and cell samples.

3. Detection principle

β-N-Acetylglucosaminidase (NAG) catalyzes the substrate to produce p-nitrophenol, which has a maximum absorption peak at approximately 400 nm. Therefore, the activity of NAG can be calculated by measuring the change in absorbance value at 400 nm.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20°C, 12 months
Reagent 2	Substrate Solution	5 mL × 1 vial	-20°C, 12 months, protect from light
Reagent 3	Chromogenic Agent	14 mL × 1 vial	-20°C, 12 months
Reagent 4	Standard	Powder × 1 vial	-20°C, 12 months, protect from light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (390-415 nm, optimum wavelength: 400 nm), Incubator (37°C)

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of measuring working solution:** Before testing, prepare sufficient measuring working solution according to the number of test wells. For example, to prepare 180 µL of measuring working solution, add 140 µL of buffer solution and 40 µL of substrate solution, and mix well. The measuring working solution should be prepared fresh and used within 1 day.
3. **Preparation of 20 mmol/L standard solution:** Dissolve one vial of standard with 1 mL of double distilled water and heat at 40°C for 3 minutes, protected from light, until dissolved. Store aliquots at -20°C for 3 months.
4. **Preparation of 0.5 mmol/L standard solution:** Dilute 20 µL of 20 mmol/L standard solution with 780 µL of double distilled water and mix well. This working solution should be prepared fresh.
5. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 0.5 mmol/L standard with double distilled water to serial concentrations. The recommended dilution gradient is: 0, 0.1, 0.15, 0.2, 0.3, 0.35, 0.4, 0.5 mmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for one month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation: 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000×g for 10 minutes at 4°C to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation: 1×10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 1×10^6 cells in 200 µL normal saline (0.9% NaCl) with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10,000×g for 10 minutes at 4°C to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

- 10% Mouse liver tissue homogenate: 3-5
- 10% Mouse kidney tissue homogenate: 3-5
- 10% Mouse heart tissue homogenate: 3-5
- 10% Mouse lung tissue homogenate: 3-5
- Rat plasma: 1
- Human serum: 1
- Bovine serum: 2-3
- 6×10^6 CHO cells: 1
- 0.6×10^6 293T/17 cells: 1

Note: The diluent is normal saline (0.9% NaCl). For dilution of other sample types, perform a pre-test to confirm the dilution factor.

8. Operating steps

1. Standard wells: Add 20 µL of different concentration solutions to standard wells. Sample wells: Add 20 µL of sample to sample wells. Control wells: Add 20 µL of sample to control wells.

2. Add 160 µL of measuring working solution to standard wells and sample wells. Add 160 µL of buffer solution to control wells.

3. Mix thoroughly with microplate reader for 5 seconds, then incubate at 37°C for 10 minutes.
4. Add 100 µL of chromogenic agent to each well.
5. Mix thoroughly with microplate reader for 5 seconds, then measure the OD value of each well at 400 nm with the microplate reader.

9. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using absolute OD value of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

1. Serum (plasma) sample:

Definition: The amount of NAG in 1 L liquid sample per 1 minute that hydrolyzes the substrate to produce 1 µmol product at 37°C is defined as 1 unit.

NAG activity (U/L) = (ΔA_400 - b) ÷ a ÷ T × f × 1000

2. Tissue sample:

Definition: The amount of NAG in 1 g tissue or cell per 1 minute that hydrolyzes the substrate to produce 1 µmol product at 37°C is defined as 1 unit.

NAG activity (U/gprot) = (ΔA_400 - b) ÷ a ÷ T × f ÷ C_{pr} × 1000

[Note]

ΔA_400: The change in OD value of sample well (ΔA_400 = A_{sample} - $A_{control}$)

T: The reaction time, 10 min

f: Dilution factor of sample before test

C_{pr}: The concentration of protein in sample, gprot/L

1000: 1 mmol/L = 1000 µmol/L

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.50	2.50	5.00
%CV	5.0	3.2	4.4

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.50	2.50	5.00
%CV	6.8	4.6	4.8

Recovery

Three samples of high, medium, and low concentrations were tested with 6 replicates each to obtain an average recovery rate of 96%.

Recovery

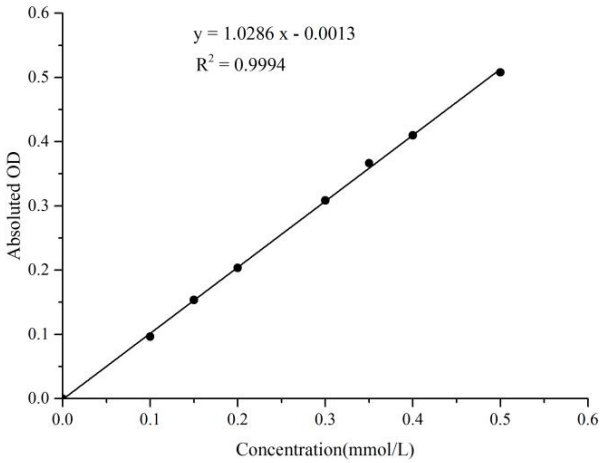
Standard	Expected Conc. (mmol/L)	Observed Conc. (mmol/L)	Recovery rate (%)
Standard 1	0.12	0.1	92
Standard 2	0.25	0.2	98
Standard 3	0.38	0.4	98

Sensitivity

The analytical sensitivity of the assay is 0.76 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only:



Standard curve

Concentration (mmol/L)	OD value	OD value	Average OD	Absolute OD
0	0.065	0.065	0.065	0.000
0.1	0.161	0.161	0.161	0.097
0.15	0.218	0.218	0.218	0.154
0.2	0.269	0.269	0.268	0.204
0.3	0.374	0.374	0.373	0.309
0.35	0.427	0.427	0.431	0.367
0.4	0.478	0.478	0.475	0.410
0.5	0.572	0.572	0.573	0.508

11. Appendix II Example Analysis

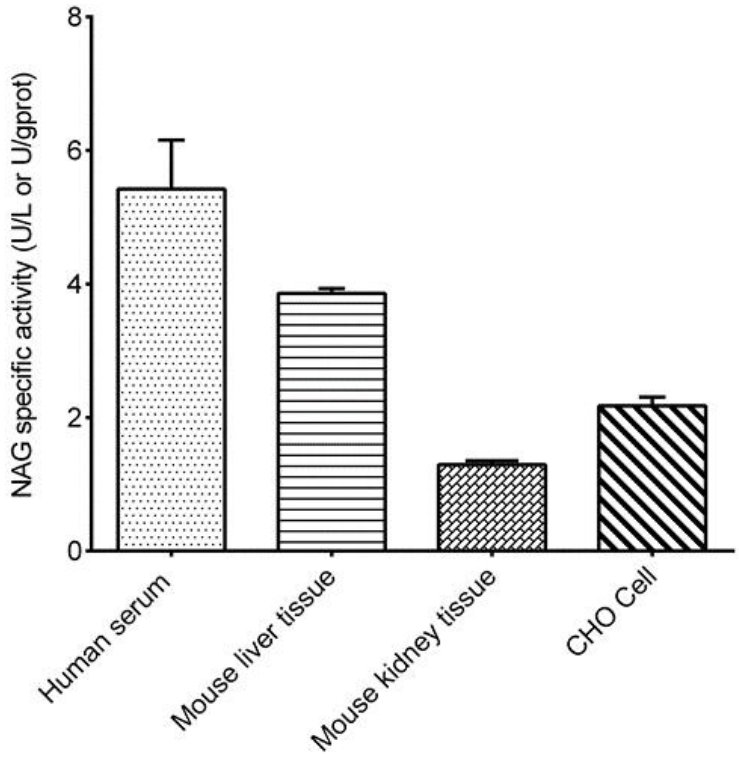
Example analysis:

For 10% mouse liver tissue homogenate, diluted 4 times, take 20 μL of diluted sample and perform the assay according to the operation protocol. The results are as follows:

Standard curve: y = 1.0286x – 0.0013. The OD value of the control well is 0.089, the OD value of the sample well is 0.178, ΔA_400 = A_{sample} - $A_{control}$ = 0.178 - 0.089 = 0.089. The concentration of protein in the sample is 9.00 gprot/L, and the calculation result is:

NAG activity (U/gprot) = (0.089 + 0.0013) ÷ 1.0286 ÷ 10 × 4 ÷ 9.00 × 1000 = 3.90 U/gprot

Detection of human serum, 10% mouse liver tissue homogenate (protein concentration: 9.00 gprot/L, diluted 4 times), 10% mouse kidney tissue homogenate (protein concentration: 9.84 gprot/L, diluted 4 times), and CHO cells (protein concentration: 0.14 gprot/L) according to the protocol yielded the following results:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.