TECHNICAL MANUAL

Neuraminidase (NA) Activity Fluorometric Assay Kit

- **SKU CODE:** MAES0324
- **SIZE:** 48 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
- Measure the fluorescence value

2. Intended use

This kit can be used to measure neuraminidase (NA) activity in animal tissue samples.

3. Detection principle

Neuraminidase (NA), also known as sialidase, is an enzyme family that hydrolyzes terminal sialic acid residues from glycolipids, oligosaccharides, and glycoproteins. It is distributed in viruses, bacteria, fungi, and vertebrate cells. In this kit, neuraminidase activity is measured by the rate of production of a fluorescent substance.

The fluorescence intensity is measured with a microplate reader at an excitation wavelength of 330 nm and an emission wavelength of 450 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	11 mL x 1 vial	20 mL x 1 vial	-20 °C, 12 months
Reagent 2	Substrate	0.3 mL x 1 vial	0.6 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 3	500 μmol/L Standard Solution	0.2 mL x 1 vial	0.4 mL x 1 vial	-20 °C, 12 months, shading light
	Black Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=330 nm/450 nm)

Reagents:

Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4).

6. Reagent preparation

1. Equilibrate all reagents to 25 °C before use.
2. **Preparation of working solution:** Before testing, prepare sufficient working solution according to the number of test wells. For example, to prepare 200 µL of working solution, thoroughly mix 5 µL of substrate with 195 µL of buffer solution. Store at 2-8 °C for up to 3 days, protected from light.
3. **Preparation of 50 µmol/L standard solution:** Before testing, prepare sufficient 50 µmol/L standard solution according to the number of test wells. For example, to prepare 1000 µL of 50 µmol/L standard solution, thoroughly mix 100 µL of 500 µmol/L standard with 900 µL of double distilled water. Store at -20 °C for up to 3 days, protected from light.
4. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 50 µmol/L standard solution with double distilled water to serial concentrations. The recommended dilution gradient is as follows: 0, 10, 20, 25, 30, 35, 40, 50 µmol/L.

7. Standard curve preparation

Item	1	2	3	4	5	6	7	8
Concentration (µmol/L)	0	10	20	25	30	35	40	50
50 µmol/L Standard (µL)	0	40	80	100	120	140	160	200
Double distilled water (µL)	200	160	120	100	80	60	40	0

8. Sample preparation

1. **Sample preparation:** 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 x g for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection. 5. Meanwhile, determine the protein concentration of supernatant (MAES0177).
2. **Dilution of sample:** The recommended dilution factor for different samples is as follows (for reference only): 10% Mouse kidney tissue homogenate: 1 10% Mouse liver tissue homogenate: 1 10% Rat liver tissue homogenate: 1 10% Mouse lung tissue homogenate: 1-2 10% Mouse heart tissue homogenate: 1 Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

9. Operating steps

1. Standard well: Add 20 µL of standard with different concentrations into the well. Sample well: Add 20 µL of sample into the well.
2. Add 180 µL of working solution into each well.
3. Mix thoroughly with microplate reader for 5 s. Measure the fluorescence intensity at the excitation wavelength of 330 nm and the emission wavelength of 450 nm, as F1. Incubate at 37 °C for 60 min and measure the fluorescence intensity at the excitation wavelength of 330 nm and the emission wavelength of 450 nm, as F2. (The standard curve is fitted to the standard well F2 values.)

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean F_2 value of the blank (Standard #①) from all standard readings. This is the absolute F_2 value.

3. Plot the standard curve by using absolute F_2 value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve ($y = ax + b$) with graph software (or Excel).

The sample:

Tissue sample:

Definition: The amount of enzyme in 1 g sample protein to catalyze the substrate to produce 1 μmol product per 1 hour at 37 °C is defined as 1 unit.

NA activity (U/g_{prot}) = (ΔF - b) ÷ a ÷ t ÷ C_{pr} × f

[Note]

ΔF: The absolute fluorescence value of sample, $\Delta F = F_2 - F_1$.

t: Reaction time, 1 h.

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in sample, g_{prot}/L.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three mouse liver tissue 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)	2.60	3.40	5.60
%CV	2.2	5.0	3.4

Inter-assay Precision

Three mouse liver tissue 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)	2.60	3.40	5.60
%CV	5.0	10.0	7.8

Recovery

Three samples of high, middle, and low concentrations were tested in parallel 6 times each to obtain an average recovery rate of 101%.

Recovery

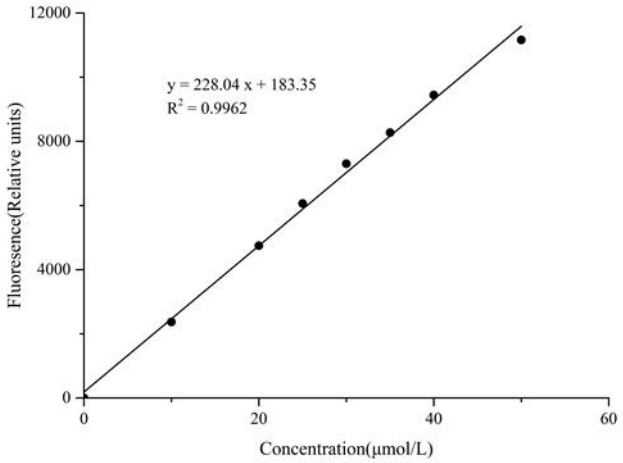
Sample	Sample 1	Sample 2	Sample 3
Expected Conc.(U/L)	2.6	10	15
Observed Conc.(U/L)	2.52	10.4	15.3
Recovery rate (%)	97	104	102

Sensitivity

The analytical sensitivity of the assay is 1.92 U/L. This was determined by adding two standard deviations to the mean fluorescence value obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the fluorescence 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 data

Concentration (μmol/L)	0	10	20	25	30	35	40	50
F2 value	16	2392	4751	6041	7246	8358	9537	10915
	9	2368	4762	6108	7385	8211	9376	11435
Average fluorescence value	13	2380	4757	6074	7315	8284	9456	11175
Absolute fluorescence value	0	2367	4744	6061	7303	8271	9443	11162

12. Appendix II Example Analysis

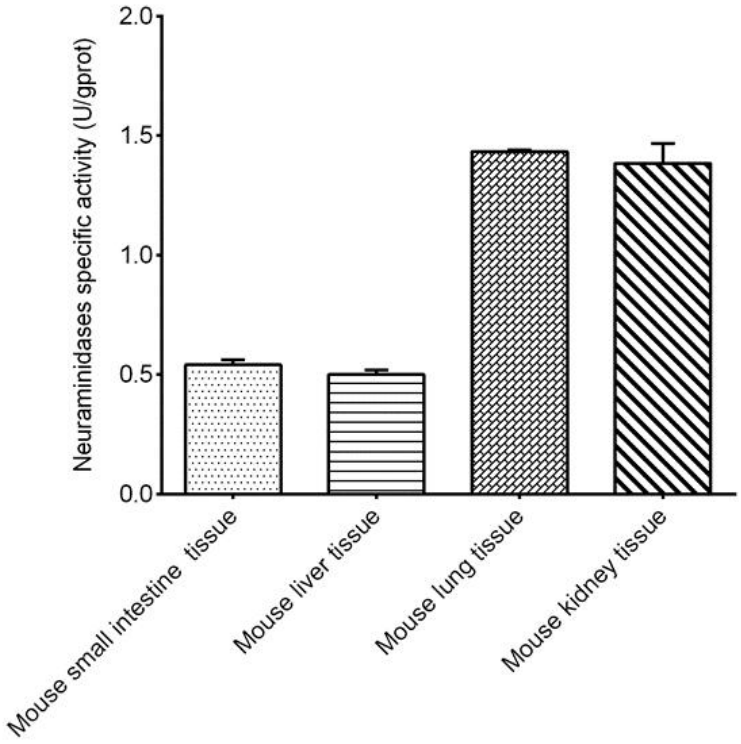
Example analysis:

Take 20 μL of 10% mouse kidney tissue homogenate and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 219.94x + 57.537, the average F_1 value of the sample well is 1901, the average F_2 value of the sample well is 5324, $\Delta F = F_2 - F_1 = 5324 - 1961 = 3363$, the concentration of protein in sample is 10.86 g_{prot}/L, and the calculation result is:

NA activity (U/g_{prot}) = (5324 - 1961 - 57.537) ÷ 219.94 ÷ 1 ÷ 10.86 = 1.38 U/g_{prot}

Detect 10% mouse small intestine tissue homogenate (the concentration of protein is 8.66 g_{prot}/L), 10% mouse liver tissue homogenate (the concentration of protein is 12.75 g_{prot}/L), 10% mouse lung tissue homogenate (the concentration of protein is 8.92 g_{prot}/L) and 10% mouse kidney tissue homogenate (the concentration of protein is 10.86 g_{prot}/L) according to the protocol, the result is as follows:



13. 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 pretest 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.