（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F083

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=330 nm/450 nm)

Detection range：1.92-7.97 U/L

Elabscience® Neuraminidase (NA) Activity Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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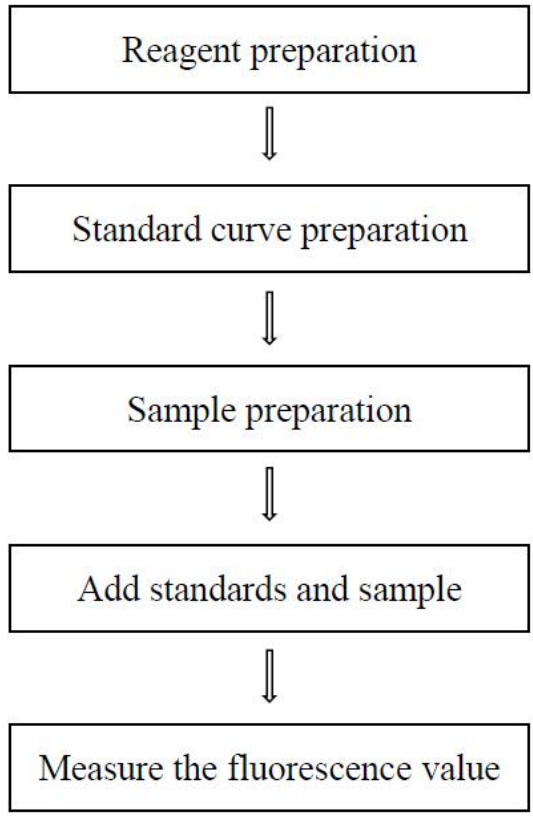
Assay summary



Intended use

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

Detection principle

Neuraminidase (NA), also known as sialase, is a family of glycolipid, oligosaccharide and glycoprotein terminal sialic acid residues that can be hydrolyzed, distributed in viruses, bacteria, fungi and fungi in vertebrate cells. In this kit, the neuraminidase activity is measured by the rate of production of fluorescent substance.

Kit components & storage

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

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

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).

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of working solution：

Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 200 μL of working solution (mix well 5 μL of substrate and 195 μL of buffer solution). Store at 2-8°C for 3 days protected from light.

③ The preparation of 50 μmol/L standard solution：

Before testing, please prepare sufficient 50 μmol/L standard solution according to the test wells. For example, prepare 1000 μL of 50 μmol/L standard solution (mix well 100 μL of 500 μmol/L standard and 900 μL of double distilled water). Store at -20°C for 3 days protected from light.

④ The 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 a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 25, 30, 35, 40, 50 μmol/L. Reference is as follows:

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

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 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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

Sample type	Dilution factor
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 do pretest to confirm the dilution factor.

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 fully with microplate reader for 5s. Measure the fluorescence intensity at the excitation wavelength of 330 nm and the emission wavelength of 450 nm, as F_1. 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 F_2. (The standard curve is fitted to the standard well in F_2 value.)

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 absoluted F_2 value.

3. Plot the standard curve by using absoluted 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.

$$\text{NA activity (U/gprot)} = (\Delta F - b) \div a \div t \div C_{pr} \times f$$

[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, gprot/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver tissue were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

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 were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.60	3.40	5.60
%CV	5.0	10.0	7.8

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 101%.

	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

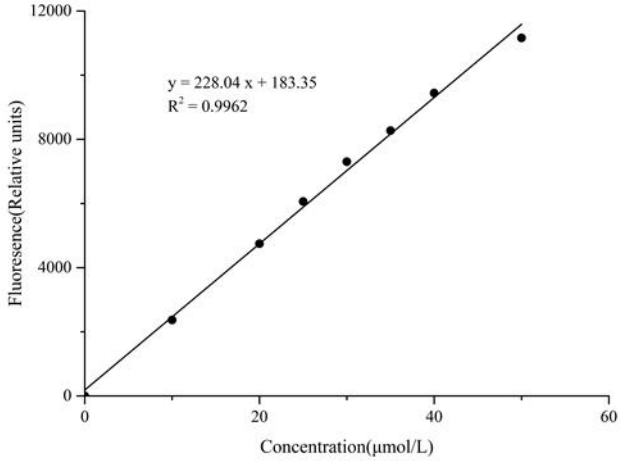
Sensitivity

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

2. 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), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	0	10	20	25	30	35	40	50
F_2 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
Absoluted fluorescence value	0	2367	4744	6061	7303	8271	9443	11162



Appendix II Example Analysis

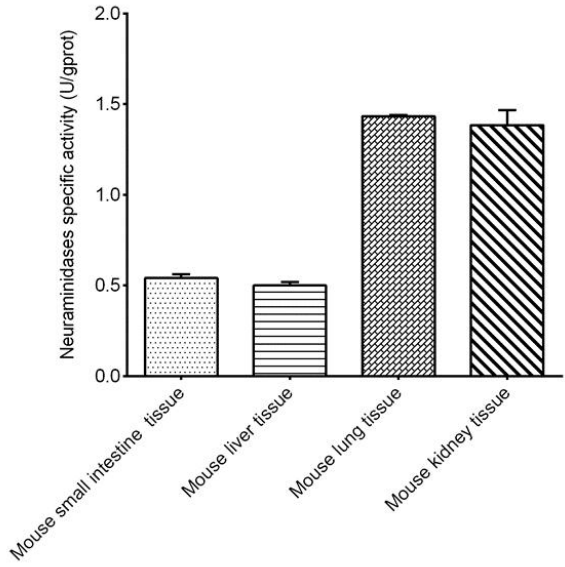
Example analysis:

Take 20 μL of 10% mouse kidney tissue homogenate and carry 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 gprot/L, and the calculation result is:

NA activity (U/gprot) = (5324 - 1961 - 57.537) ÷ 219.94 ÷ 1 ÷ 10.86 = 1.38 U/gprot

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



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.