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

Catalog No：E-BC-D004

Specification：96T

Measuring instrument：Fluorescence Microplate Reader

（Ex/Em=330nm/450nm）

Elabscience® Neuraminidases (NA) Inhibitor Screening 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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Intended use

This kit is used for the determination of the inhibitory effect of neuraminidases (NA) inhibitors.

Detection principle

Neuraminidases, also known as sialidase, are a family of exoglycans that hydrolyse glycolipids, oligosaccharides and sialic acid residues at the end of glycoproteins. NA is distributed in viruses, bacteria, fungi and vertebrate cells. Viral neuraminidase is one of the important targets of some influenza drugs. Human neuraminidase plays a role in a variety of signaling pathways and is associated with a variety of diseases such as neurodegenerative diseases, cancer, and cardiovascular and cerebrovascular diseases. Therefore, it is important to develop NA inhibitors. The detection principle of this kit is that NA decomsolves the substrate to release fluorescent substances, and the activity can be inhibited by adding NA inhibitor. The inhibition ability of inhibitor can be judged by the fluorescence value.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	25 mL ×1 vial	-20ºC, 12 months
Reagent 2	Substrate	0.6 mL ×1 vial	-20ºC, 12 months, shading light
Reagent 3	Enzyme Reagent	Powder ×1 vial	-20ºC, 12 months, shading light
Reagent 4	1 mmol/L Oseltamivir Acid	0.24 mL ×1 vial	-20ºC, 12 months, shading light
	Black Microplate		No requirement
	Plate Sealer		

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), Incubator (37°C)

Reagent preparation

① Keep enzyme reagent on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of working solution：

For each well, prepare 100 μL of working solution (mix well 5 μL of substrate and 95 μL of buffer solution). Store at 2-8 ℃ for 3 days protected from light.

③ The preparation of enzyme working solution:

Dissolve one vial of enzyme reagent with 240 μL of buffer solution, mix well to get concentrated enzyme working solution. Before testing, please prepare sufficient enzyme working solution according to the test wells. For example, prepare 253 μL of enzyme working solution (mix well 3 μL of concentrated enzyme working solution and 250 μL of buffer solution). Store at 2-8 ℃ for 1 day protected from light. The concentrated enzyme working solution can be stored at -20 ℃ for 14 days, and avoid repeated freeze/thaw cycles is advised.

④ The preparation of inhibitor working solution:

Before testing, please prepare sufficient inhibitor working solution according to the test wells. For example, prepare 50 μL of inhibitor working solution (mix well 5 μL of 1 mmol/L oseltamivir acid and 45 μL of buffer solution). Store at 2-8 ℃ for 3 days protected from light. The 1 mmol/L oseltamivir acid can be stored at -20 ℃ for 1 month, and avoid repeated freeze/thaw cycles is advised. (This reagent is a NA specific inhibitor and can be used according to

the situation.)

Sample preparation

It is recommended to use DMSO as a sample solvent for compounds.

The key points of the assay

① Protect from light during reagent preparation.
② After adding sample, it is recommend to mix fully with microplate reader.
③ The reaction will start immediately after adding substrate. It is recommended to use multichannel pipeter to shorten the time and reduce the error between wells.

Operating steps

① Blank well: Add 80 μL of buffer solution to the wells;
Control well: Add 80 μL of enzyme working solution to the wells;
Sample well: Add 80 μL of enzyme working solution to the wells.
② Add 20 μL of sample solvent into blank and control wells;
Add 20 μL of sample into samples wells.
③ Mix fully with microplate reader for 3 s and incubate at 37°C for 10 min.
④ Add 100 μL of working solution to each well.
⑤ Mix fully with microplate reader for 3 s and incubate at 37°C for 30 min.
Measure the fluorescence intensity of each well at the excitation wavelength of 330 nm and the emission wavelength of 450 nm.

Calculation

$$\text{Inhibition rate (\%)} = (F_{control} - F_{sample}) \div (F_{control} - F_{blank}) \times 100\%$$

[Note]

$F_{control}$: The fluorescence intensity of control well.

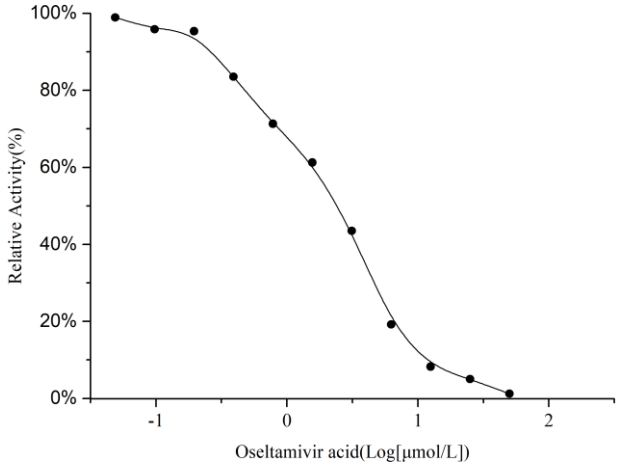
F_{sample}: The fluorescence intensity of sample well.

F_{blank}: The fluorescence intensity of blank well.

Appendix I Performance Characteristics

Inhibition curve

Effect diagram of neuraminidases (NA) inhibitor screening kit for detecting NA inhibitor oseltamivir acid.



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.