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

Catalog No：E-BC-D007

Specification：96T

Measuring instrument：Fluorescence Microplate Reader

Elabscience® Dipeptidyl Peptidase IV (DPP4) 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
Tel: 1-832-243-6086
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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 dipeptide base peptidase 4(DPP4) inhibitors.

Detection principle

Dipeptide base peptidase 4 (Dipeptidyl peptidase 4, DPP4), also known as CD26, is a kind of serine protease, can decompose the peptide chain N the second place, proline and alanine residue peptide bond. DPP4 can quickly decompose enterotropin in living organisms, which can stabilize insulin levels and promote a decrease in blood sugar levels in the body. The detection principle of this kit is that DPP4 can decompose the substrate and release fluorescent substance AMC. The addition of DPP4 inhibitor can inhibit the enzyme activity, and the inhibitory ability of the inhibitor can be determined by the fluorescence value.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	25 mL ×1 vial	-20 ℃, 12 months
Reagent 2	Enzyme Reagent	Powder ×4 vials	-20 ℃, 12 months, shading light
Reagent 3	Substrate	1.2 mL ×2 vials	-20 ℃, 12 months, shading light
Reagent 4	1 mmol/L Sitagliptin	0.3 mL ×2 vials	-20 ℃, 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=360 nm/460 nm), Incubator

Reagents:

Double distilled water

Reagent preparation

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

② Preparation of enzyme working solution:
Dissolve one vial of enzyme reagent with 0.6 mL of double distilled water, mix well to dissolve. Store at 2-8 ℃ for 1 day protected from light.

③ Preparation of reaction working solution:
For each well, prepare 170 μL of reaction working solution (mix well 17 μL of substrate and 153 μL of buffer solution). The reaction working solution should be prepared on spot. Store at 2-8 ℃ for 1 day protected from light. It is recommended to aliquot substrate and storage at -20 ℃, and avoid repeated freeze/thaw cycles is advised.

④ Preparation of 100 μmol/L Sitagliptin 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 sitagliptin and 45 μL of buffer solution). The inhibitor working solution should be prepared on spot. Store at 2-8 ℃ for 3 days protected from light. It is recommended to aliquot 1 mmol/L sitagliptin and storage at -20 ℃, and avoid repeated freeze/thaw cycles is advised.
Note: This reagent is a DPP4 specific inhibitor and can be used according to the situation.

Sample preparation

Sample preparation

The compound sample should be dissolved in a suitable solvent, and it is recommended to use DMSO as the solvent to prepare a stock solution. Dilute to the required concentration using double distilled water for use. (High concentration of DMSO will affect the enzyme activity, so it is recommended that the concentration of DMSO in the solution of the compound should be less than 10%)

The key points of the assay

① Pay attention to avoiding light during the preparation process of reagent.

② After adding the sample, the microplate can be slightly shaken to mix the reaction reagents evenly.

③ The reaction will start immediately after adding the substrate. It is recommended to use multichannel pipeter to shorten the time and reduce the error between wells.

Operating steps

① Blank well: Add 20 μL of buffer solution to the corresponding wells.

Control well: Add 20 μL of enzyme working solution to the corresponding wells.

Sample well: Add 20 μL of enzyme working solution to the corresponding wells.

② Add 30 μL of sample solvent to blank well and control well. Add 30 μL of sample to sample well.

③ Mix fully with microplate reader for 3 s and incubate at 37℃ for 10 min.

④ Add 170 μL of reaction working solution into each well.

⑤ Incubate at 37℃ for 30 min. Measure the fluorescence intensity of each well at

the excitation wavelength of 360 nm and the emission wavelength of 460 nm.

Calculation

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

[Note]

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

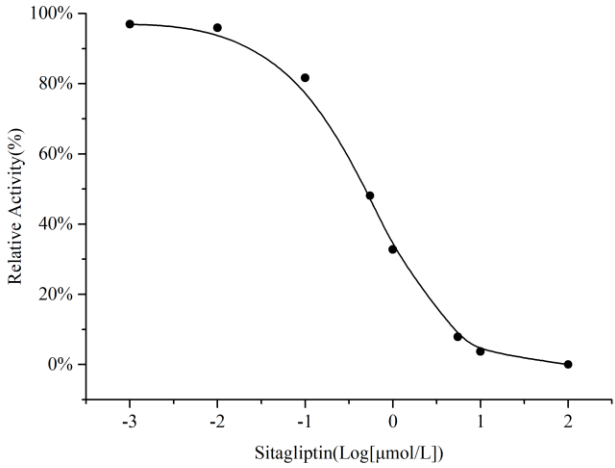
F_{sample}: The fluorescence intensity of sample well.

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

Appendix I Performance Characteristics

Inhibition curve

Effect diagram of DPP4 inhibitor screening kit for detecting DPP4 inhibitor Sitagliptin.



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.