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

Catalog No：E-BC-F053

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=360 nm/460 nm)

Detection range：0.92-3.77 U/L

Elabscience® Dipeptidyl Peptidase IV(DPP4) 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
Tel: 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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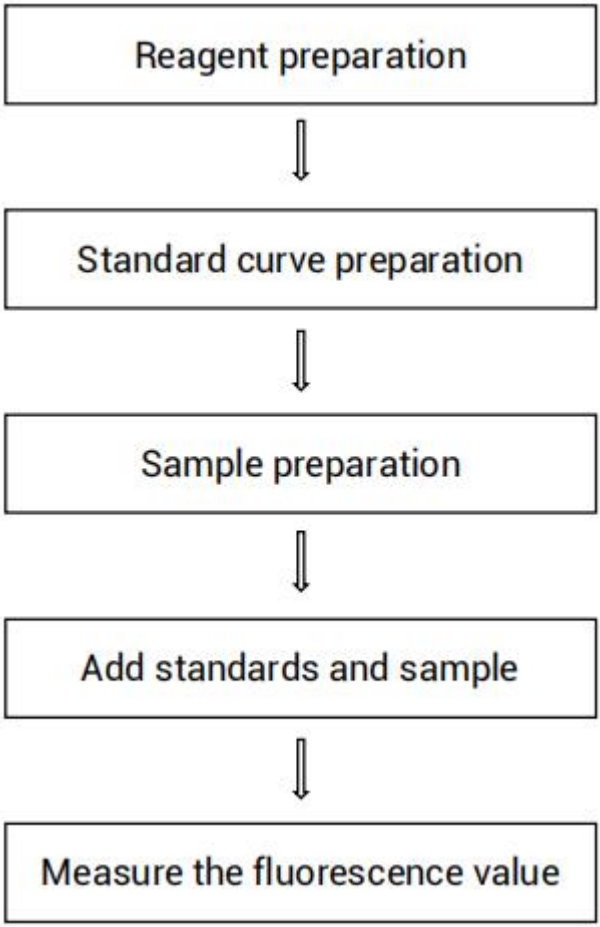
Assay summary



Intended use

This kit can be used to measure dipeptidyl deptidase IV (DPP4) activity in serum, plasma, and tissue and cell samples.

Detection principle

Dipeptidyl Peptidase IV (DPP4), also known as CD26, is a kind of serine protease, can decompose the second N-terminal proline or alanine residue of the peptide chain. In organisms, dipeptidyl peptidase can rapidly decompose incretin, which can stabilize insulin level and promote the reduction of blood glucose level in organisms. The detection principle of this kit is that DPP4 can decompose the substrate and release the fluorescent substance AMC, and the activity of this enzyme can be inhibited by adding DPP4 inhibitor. The activity of DPP4 could be calculated by the difference of fluorescence value before and after inhibition.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	-20℃, 12 months
Reagent 2	Postive Control	Powder ×1 vial	-20℃, 12 months shading light
Reagent 3	Substrate	1.2 mL × 2 vials	-20℃, 12 months shading light
Reagent 4	Inhibitors	0.3 mL × 2 vials	-20℃, 12 months shading light
Reagent 5	1 mmol/L Standard	1 mL × 1 vial	-20℃, 12 months shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

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:

Vortex mixer, 37℃incubator, Centrifuge, Fluorescence microplate reader (Ex/Em=360 nm/460 nm)

Reagent preparation

① Keep postive control on ice for use. Equilibrate all the reagents to room temperature before use.

② The preparation of positive working solution (DPP4):
Dissolve one vial of postive control with 0.2 mL of double distilled water, mix well. Store at 2-8°C for 1 day protected from light.
(Positive control well and positive sample well are selective testing to j udge whether the reagent properties are normal.)

③ The preparation of reaction working solution:
For each well, prepare 170 uL of reaction working solution (mix well 17 μL of substrate working solution and 153 μL of buffer solution). Store at 2-8°C for 1 day protected from light. The substrate can be aliquoted storage at -20°C, and avoid repeated freeze/thaw cycles is advised.

④ The preparation of inhibition working solution:
Before testing, please prepare sufficient inhibition working solution according to the test wells. For example, prepare 200 μL of inhibition working solution (mix well 5 μL of inhibition and 195 μL of buffer solution). Store at 2-8°C for 1 day protected from light. Aliquoted

storage at -20°C, and avoid repeated freeze/thaw cycles is advised.

⑤ The preparation of 100 μmol/L standard solution:

Dilute 100 μL of 1 mmol/L standard with 900 μL of buffer solution and mix fully.Store at 2-8°C for 3 days protected from light. The 1 mmol/L standard can be aliquoted storage at -20°C, and avoid repeated freeze/thaw cycles is advised.

⑥ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 μmol/L standard with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 20, 40, 50, 60, 70, 80, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**20**	**40**	**50**	**60**	**70**	**80**	**100**
100 μmo/L standard (μL)	0	40	80	100	120	140	160	200
Buffer solution (μL)	200	160	120	100	80	60	40	0

Sample preparation

① 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 a 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 380 μL buffer solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min 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)

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 buffer solution with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min 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
5% Mouse kidney tissue homogenate	4-5
5% Mouse lung tissue homogenate	4-5
5% Mouse heart tissue homogenate	4-5
5% Rat liver tissue homogenate	4-5
5% Rat brain tissue homogenate	4-5
Porcine serum	4-5
Horse serum	4-5
Dog serum	4-5
Female chicken serum	4-5
Macaca fascicularis serum	4-5
5.0×10^6 HL-60 cell	1
4.1×10^6 293T cell	1

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

After adding samples and inhibition working solution, mix fully with microplate reader to reduce experimental errors.

Operating steps

① Standard well: add 20 μL of standard with different concentrations into the standard wells.
Sample well: add 20 μL of sample into the corresponding wells.
Control well: add 20 μL of sample into the corresponding wells.
Positive control well: add 20 μL of positive working solution into the corresponding wells.
Positive sample well: add 20 μL of positive working solution into the corresponding wells.

② Standard well: add 200 μL of buffer solution into the corresponding wells.
Control well, positive control well: Add 30 μL of buffer solution into the corresponding wells.
Sample well, positive sample well: Add 30 μL of inhibition working solution into the corresponding wells.

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 control well, sample well, positive control well and positive sample well.

⑤ Measure the fluorescence intensity at the excitation wavelength of 360 nm and the emission wavelength of 460 nm, record as F_1. Incubate at 37℃ for 30 min, measure the fluorescence intensity at the excitation wavelength of 360 nm and the emission wavelength of 460 nm, record as F_2, $\triangle F = F_2 - F_1$.(Standard wells only need to measure F_2).

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted fluorescence 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:

1. Serum (plasma) sample:

Definition: The amount of enzyme in 1 L of serum or plasma that catalyze the production of 1 μmol AMC per minute at 37℃ is defined as 1 unit.

$$\text{DPP4 activity (U/L)} = (\Delta F_{control} - \Delta F_{sample}) \div a \div t \times f$$

2. Tissue and cell samples:

Definition: The amount of enzyme in 1 g of tissue or cell protein that catalyze the production of 1 μmol AMC per minute at 37℃ is defined as 1 unit.

$$\text{DPP4 activity (U/L)} = (\Delta F_{control} - \Delta F_{sample}) \div a \div t \div C_{pr} \times f$$

[Note]

ΔF_{sample}: The absolute fluorescence value of sample well, $F_2 - F_1$

$\Delta F_{control}$: The absolute fluorescence value of control well, $F_2 - F_1$

t: the reaction time, 30 min.

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 human serum samples 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)	1.00	2.50	3.00
%CV	1.0	0.4	0.4

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.00	2.50	3.00
%CV	4.5	6.2	6.4

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 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	25	55	75
Observed Conc. (μmol/L)	24.8	55.0	75.8
Recovery rate (%)	99	100	101

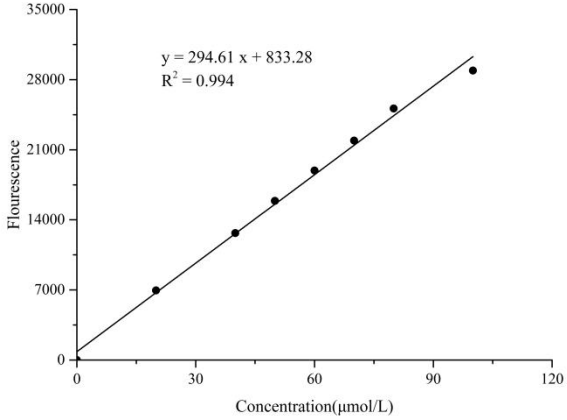
Sensitivity

The analytical sensitivity of the assay is 0.92 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**	**20**	**40**	**50**	**60**	**70**	**80**	**100**
Fluorescence value	16	6862	12505	15781	18403	21563	24781	28544
	20	7082	12876	16059	19481	22312	25503	29309
Average fluorescence value	18	6972	12691	15920	18942	21938	25142	28927
Absoluted fluorescence value	0	6954	12673	15902	18924	21920	25124	28909



Appendix Π Example Analysis

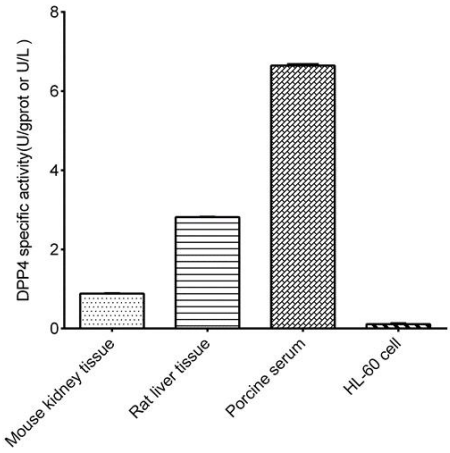
Example analysis：

For 5% mouse kidney tissue, dilute for 4 times, take 20 μL for detection, and carry the assay according to the operation table. The results are as follows:

standard curve: y = 294.61 x + 833.29, the average fluorescence value of the control F1 is 7917, the average fluorescence value of the sample F1 is 7498.5. After 30 mins, the average fluorescence value of the control F2 is 15582.5, the average fluorescence value of the sample F2 is 10508.5, △Fsample = 10508.5 - 7498.5 = 3010, △Fcontrol = 15582.5 - 7917 = 7665.5, the concentration of protein in sample is 3.97 gprot/L, and the calculation result is:

DPP4 activity (U/gprot) = (7665.5 - 3010) ÷ 294.61 ÷ 30÷ 3.97 × 4 = 0.53 U/gprot

Detect 5% mouse kidney tissue homogenate (the concentration of protein is 3.97 gprot/L, dilute for 4 times), 5% rat liver tissue homogenate (the concentration of protein is 4.72 gprot/L, dilute for 4 times), porcine serum (dilute for 4 times) and HL-60 cell (the concentration of protein is 0.68 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.