



**PRODUCT INFORMATION &
MANUAL**

**DPPIV/CD26 Activity Assay
Kit (Fluorometric)
*NBP3-24487***

For research use only.
Not for diagnostic or therapeutic
procedures.

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Novus kits are guaranteed for 6 months from date of receipt

DPPIV/CD26 Activity Assay Kit (Fluorometric)

Catalog No: NBP3-24487

Method: Fluorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Fluorimetric Microplate Reader

Sensitivity: 0.92 U/L

Detection range: 0.92-3.77 U/L

Average intra-assay CV (%): 0.6

Average inter-assay CV (%): 5.7

Average recovery rate (%): 100

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲ Intended use

This kit can be used to measure dipeptidyl peptidase 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	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	-20°C , 12 months
Reagent 2	Postive Control	Powder × 1 vial	-20°C , 12 months, shading light
Reagent 3	Substrate	1.2 mL × 2 vials	-20°C , 12 months, shading light
Reagent 4	Inhibitor	0.3 mL × 2 vials	-20°C , 12 months, shading light
Reagent 5	1 mmol/L Standard	1 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.

▲ Materials prepared by users

Instruments

Vortex mixer, 37°C incubator, Centrifuge, Fluorescence microplate reader

(Ex/Em=360 nm/460 nm).

▲ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

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

Pre-assay preparation

▲ Reagent preparation

1. Place the reagent 2 on ice box for use. Bring other reagents to room temperature before use.
2. **Preparation of positive working solution (DPP4):**
Dissolve a vial of reagent 2 with 0.2 mL of double distilled water and mix fully. It can be stored at 2-8°C with shading light for 1 day.
3. **Preparation of reaction working solution:**
Mix the reagent 3 and reagent 1 at a ratio of 1: 9. Prepare the needed amount of fresh solution before use. The prepared solution can be stored at 2-8°C with shading light for 1 day. The reagent 3 can be divided into smaller packages and stored at -20°C to avoid repeated freezing and thawing.
4. **Preparation of inhibition working solution:**
Mix the reagent 4 and reagent 1 at a ratio of 1: 39. Prepare the needed amount of fresh solution before use. The prepared solution can be stored at 2-8°C with shading light for 1 day. The reagent 4 can be divided into smaller packages and stored at -20°C to avoid repeated freezing and thawing.
5. **Preparation of 100 µmol/L standard solution:**
Mix the reagent 5 and reagent 1 at a ratio of 1: 9 fully. Prepare the needed amount of fresh solution before use. The prepared solution can be stored at 2-8°C with shading light for 3 days. The reagent 5 can be divided into smaller packages and stored at -20°C to avoid repeated freezing and thawing.

▲ Sample preparation

1. Serum and plasma samples:

Detect the sample directly.

2. Tissue sample:

Accurately weigh the tissue, add 9 times the volume of reagent 1 according to the ratio of Weight (g): Volume (mL) =1:19. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min. If the supernatant is turbidity after centrifugation, centrifuge the supernatant repeated before use. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

3. Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): reagent 1 (μL) =1: 200. Sonicate or mechanical homogenate in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

▲ Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.92-3.77 U/L).

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 brain tissue homogenate	4-5
5% Mouse heart tissue homogenate	4-5
5% Mouse liver 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 reagent 1.

Assay protocol

▲ Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40 control wells; S1'- S40' sample wells.

▲ Detailed operation steps

1. The preparation of standard curve

Dilute 100 $\mu\text{mol/L}$ standard with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0, 20, 40, 50, 60, 70, 80, 100 $\mu\text{mol/L}$. Reference is as follows:

Number	Standard concentrations ($\mu\text{mol/L}$)	100 $\mu\text{mol/L}$ standard (μL)	Reagent 1 (μL)
A	0	0	200
B	20	40	160
C	40	80	120
D	50	100	100
E	60	120	80
F	70	140	60
G	80	160	40
H	100	200	0

2. The measurement of samples

(1) **Standard well:** Add 20 μL of standard with different concentrations to the corresponding wells.

Sample well: Add 20 μL of sample to the corresponding wells.

Control well: Add 20 μL of sample to the corresponding wells.

Positive control well: Add 20 μL of positive working solution to the corresponding wells.

Positive sample well: Add 20 μL of positive working solution to the corresponding wells.

(2) **Standard well:** Add 200 μL of reagent 1 to the corresponding wells.

Control well, Positive control well: Add 30 μL of reagent 1 to the corresponding wells.

Sample well, Positive sample well: Add 30 μL of inhibition working solution to the corresponding wells.

(3) Mix fully with microplate reader for 3 s and incubate at 37°C for 10 min.

(4) Add 170 μL of reaction working solution to 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°C 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 , $\Delta F = F_2 - F_1$. (Standard wells only need to measure F_2).

▲ Summary operation table

	Standard well	Control well	Sample well	Positive control well	Positive sample well
Standard with different concentrations (μL)	20				
Sample (μL)		20	20		
Positive working solution (μL)				20	20
Reagent 1 (μL)	200	30		30	
Inhibition working solution (μL)			30		30
Mix fully and incubate at 37°C for 10 min.					
Reaction working solution (μL)		170	170	170	170
Measure the fluorescence intensity, record as F_1 . Incubate at 37°C for 30 min, measure the fluorescence intensity, record as F_2 , $\Delta F = F_2 - F_1$. (Standard wells only need to measure F_2).					

▲ Calculation

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the F value of sample. The standard curve is: $y = ax + b$.

1. For Serum/plasma:

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

$$\text{DPP4 activity (U/L)} = (F_{\text{Control}} - F_{\text{Sample}} - b) \div a \div t \times f$$

2. Tissue ana cell:

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°C is defined as 1 unit.

$$\text{DPP4 activity (U/gprot)} = (F_{\text{Control}} - F_{\text{Sample}} - b) \div a \div t \div C_{\text{pr}} \times f$$

Note:

y: $F_{\text{Standard}} - F_{\text{Blank}}$ (F_{Blank} is the fluorescence value when the standard concentration is 0)

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

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

$\Delta F_{\text{control}}$: 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 Data

▲ 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: $y = 294.61x + 833.29$, the average fluorescence value of the control F_1 is 7917, the average fluorescence value of the sample F_1 is 7498.5. After 30 mins, the average fluorescence value of the control F_2 is 15582.5, the average fluorescence value of the sample F_2 is 10508.5, $\Delta F_{\text{sample}} = 10508.5 - 7498.5 = 3010$, $\Delta F_{\text{control}} = 15582.5 - 7917 = 7665.5$, the concentration of protein in sample is 3.97 gprot/L, and the calculation result is:

$$\begin{aligned} \text{DPP4 activity (U/gprot)} &= (7665.5 - 3010 - 833.29) \div 294.61 \div 30 \div 3.97 \times 4 \\ &= 0.43 \text{ U/gprot} \end{aligned}$$