TECHNICAL MANUAL

DPP4 Activity Fluorometric Assay Kit

- **SKU CODE:** MAES0237
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Add reaction working solution
- Measure fluorescence intensity

2. Intended use

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

3. Detection principle

Dipeptidyl Peptidase IV (DPP4), also known as CD26, is a serine protease that can decompose the second N-terminal proline or alanine residue of peptide chains. In organisms, dipeptidyl peptidase can rapidly decompose incretin, which helps stabilize insulin levels and promotes blood glucose reduction in organisms.

The detection principle of this kit is based on DPP4's ability to decompose the substrate and release a fluorescent substance. The activity of this enzyme can be inhibited by adding a DPP4 inhibitor. The activity of DPP4 is calculated from the difference in fluorescence values before and after inhibition.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	60 mL x 1 vial	-20°C, 12 months
Reagent 2	DPP4	Powder x 1 vial	-20°C, 12 months, protect from light
Reagent 3	Substrate	1.2 mL x 2 vials	-20°C, 12 months, protect from light
Reagent 4	Inhibitor	0.3 mL x 2 vials	-20°C, 12 months, protect from light

Item	Component	Size (96 T)	Storage
Reagent 5	1 mmol/L Standard	1 mL x 1 vial	-20°C, 12 months, protect from light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments: Vortex mixer, 37°C incubator, centrifuge, fluorescence microplate reader (Ex/Em = 360 nm/460 nm)

6. Reagent preparation

1. Keep positive control on ice for use. Allow all reagents to equilibrate to room temperature before use.
2. **Preparation of positive working solution:** Dissolve one vial of DPP4 with 0.2 mL of double-distilled water and mix well. Store at 2-8°C for 1 day, protected from light. (Positive control well and positive sample well are selective tests to judge whether the reagent properties are normal. This kit includes an additional reagent set for positive detection, which does not reduce the number of testable samples.)
3. **Preparation of reaction working solution:** For each well, prepare 170 µL 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 and stored at -20°C; avoid repeated freeze/thaw cycles.
4. **Preparation of inhibition working solution:** Before testing, 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. Can be aliquoted and stored at -20°C; avoid repeated freeze/thaw cycles.
5. **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 and stored at -20°C; avoid repeated freeze/thaw cycles.
6. **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 create a serial concentration. The recommended dilution gradient is as follows: 0, 20, 40, 50, 60, 70, 80, 100 μmol/L.

7. 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 10,000 x 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 (MAES0177).

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation 1 x 10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 1 x 10^6 cells in 200 μL buffer solution with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10,000 x 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 (MAES0177).

Dilution of sample: The recommended dilution factor for different samples is as follows (for reference only). The diluent is buffer solution. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

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

9. 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 seconds and incubate at 37°C 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 F1. 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 F2. ΔF = F2 - F1. (Standard wells only need to measure F2.)

10. 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 absolute fluorescence value.

3. Plot the standard curve by using absolute 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 catalyzes the production of 1 µmol AMC per minute at 37°C is defined as 1 unit.

DPP4 activity U/L = (ΔF_control - ΔF_sample) ÷ a ÷ t × f

2. Tissue and cell samples:

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

DPP4 activity U/gprot = (ΔF_control - ΔF_sample) ÷ a ÷ t ÷ C_{pr} × f

[Note]

ΔF_sample: The absolute fluorescence value of sample well, F_2 - F_1

ΔF_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

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Inter-assay Precision

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

Recovery

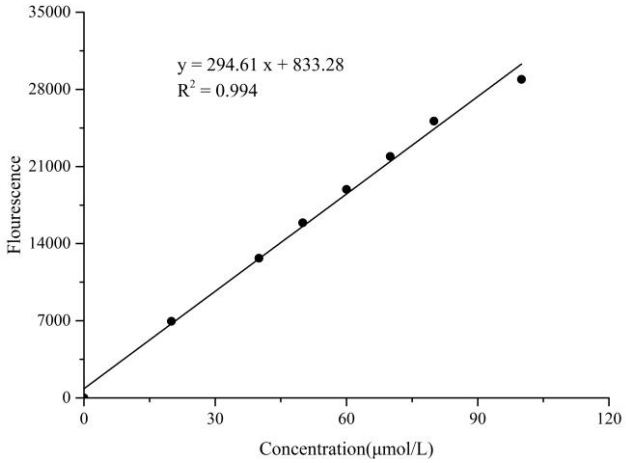
Three samples of high concentration, middle concentration, and low concentration were tested with each concentration run 6 times in parallel to obtain an average recovery rate of 100%.

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.

Standard curve data

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), the standard curve and data are provided below for reference only.



11. Appendix II Example Analysis

Example analysis:

For 5% mouse kidney tissue, dilute 4 times, take 20 μL for detection, and carry out 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 min, the average fluorescence value of the control F_2 is 15582.5

The average fluorescence value of the sample F_2 is 10508.5

ΔF_sample = 10508.5 - 7498.5 = 3010

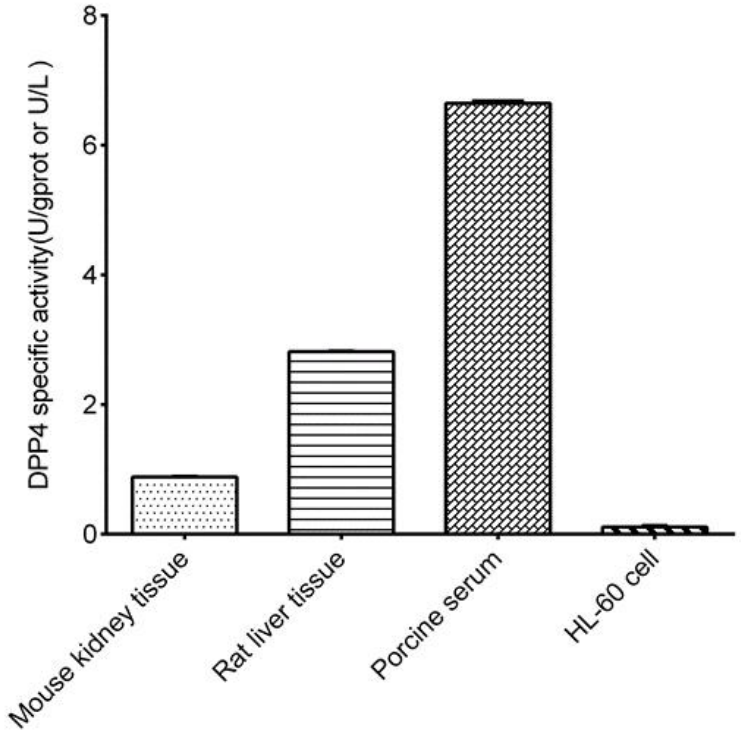
ΔF_control = 15582.5 - 7917 = 7665.5

The concentration of protein in sample is 3.97 gprot/L

Calculation result:

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

Detection of 5% mouse kidney tissue homogenate (protein concentration 3.97 gprot/L, diluted 4 times), 5% rat liver tissue homogenate (protein concentration 4.72 gprot/L, diluted 4 times), porcine serum (diluted 4 times), and HL-60 cell (protein concentration 0.68 gprot/L) according to the protocol:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.