Amplite® Fluorimetric DPP4 Activity Assay Kit

Catalog number: 11323
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 11323)
Component A: DPP4 Assay Buffer	Freeze (< -15 °C), Minimize light exposure	25 mL
Component B: DPP4 Substrate	Freeze (< -15 °C)	0.25 mL
Component C: DPP4 Positive Control	Freeze (< -15 °C)	20 µL
Component D: AMC Standard	Freeze (< -15 °C)	0.1 mL
Component E: DPP4 Inhibitor (Sitagliptin)	Freeze (< -15 °C)	50 µL

OVERVIEW

The Amplite® Fluorimetric DPP4 Activity Assay Kit provides a simple, quick, and direct protocol for measuring DPP4 Activity. This assay leverages the DPP4-mediated cleavage of a non-fluorescent substrate, resulting in a fluorescent product with Ex/Em = 360/460 nm, respectively. The fluorescence intensity generated is directly proportional to the DPP4 activity in the sample, making it suitable for detecting DPP4 in various biological matrices such as cellular lysates, tissue extracts, and serum. Additionally, the assay is compatible with high-throughput screening systems. One unit (U) is the amount of enzyme that catalyzes the reaction of 1 µmol of substrate per minute. Dipeptidyl peptidase-4, also known as DPP4, CD26, ADCP2, DPP, is a transmembrane glycoprotein belonging to the prolyl oligopeptidase family. It is a serine exopeptidase that cleaves X-proline and X-Alanine residues from the N-terminal ends of polypeptides. DPP4 is involved in several physiological processes, including glucose metabolism through the regulation of glucagon-like-peptide (GLP-1), immune regulation by acting as a receptor on many immune cells, signal transduction as a transmembrane protein responsive to growth factors and chemokines, and tumor suppression via immune modulation. DPP4 inhibitors are being used as a treatment for type-2 diabetes.

AT A GLANCE

Important Note

Thaw all the kit components at room temperature before starting the experiment.

Protocol Summary

1. Prepare the test samples, DPP4 Positive Control, and the serially diluted AMC standards (50 µL).
2. Add the DPP4 working solution (50 µL).
3. Incubate at room temperature for 10-30 minutes.
4. Monitor the fluorescence intensity at Ex/Em=360/460 nm, cutoff=435 nm.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	435 nm
Emission	460 nm
Excitation	360 nm
Recommended plate	Solid black or black plate with clear bottom

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11323

AMC Standard

Add 5 µL of 1 mM AMC standard solution to 495 µL of DPP4 Assay Buffer (Component A) to prepare a 10 µM AMC standard solution (STD7). Then, take 300 µL of STD7 and perform 1:2 serial dilutions in of DPP4 Assay Buffer (Component A) to create a series of AMC standards from STD7 to STD1.

PREPARATION OF WORKING SOLUTION

DPP4 Working Solution

1. Add 0.25 mL of the DPP4 Substrate (Component B) to 5 mL of the DPP4 Assay Buffer (Component A), and mix well.

   Note: This DPP4 working solution should be freshly prepared before each experiment and protected from light. A 5 mL solution is enough for 100 tests. Please prepare the necessary amount of DPP4 working solution based on this proportion.

5X DPP4 Inhibitor Working Solution

1. Add 2.5 µL of DPP4 Inhibitor (Sitagliptin) (Component E) to 500 µL of DPP4 Assay Buffer (Component A) to create a 5X DPP4 Inhibitor Working Solution.

DPP4 Positive Control

1. Prepare one or more DPP4 positive controls along with your test sample. The suggested concentration for these controls is between 0.5 µg/mL and 0.05 µg/mL in the DPP4 Assay Buffer (Component A). For example, to make a 0.5 µg/mL DPP4 positive control, add 2.5 µL of the DPP4 Positive Control Stock Solution to 250 µL of the DPP4 Assay Buffer (Component A).

Test Samples

1. Tissue and cells can be homogenized in the DPP4 Assay Buffer (Component A). After homogenization, centrifuge the sample at 13,000xg for 10 minutes to remove insoluble material. Serum samples can be directly added to the wells. Adjust the final volume of all samples to 50 µL using DPP4 Assay Buffer.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of AMC standards and test samples in a 96-well clear bottom microplate. (STD = AMC Standards (STD7-STD1, 10 to 0.0781 µM), BL= Blank Control, TS = Test Samples.)

BL	BL	Positive Control	Inhibitor Control	TS
STD 1	STD 1	...	...	...

STD 2	STD 2	...	...	...
STD 3	STD 3			
STD 4	STD 4			
STD 5	STD 5			
STD 6	STD 6			
STD 7	STD 7			

Table 2. Reagent composition for each well.

Well	Volume	Reagent
STD 1 -STD 7	50 µL	AMC Serial Dilutions (0.0781-10 µM)
BL	50 µL	DPP4 Assay buffer
DPP4 Positive Control	50 µL	40 µL DPP4 Positive Control + 10 µL DPP4 Assay buffer
DPP4 Inhibitor Control	50 µL	40 µL DPP4 Positive Control + 10 µL 5X DPP4 Inhibitor Working Solution
TS	50 µL	Cell content

1. Prepare the AMC standards (STD1-7), blank controls (BL), DPP4 Positive Control, DPP4 Inhibitor Control, and test samples (TS) according to the layout provided in Tables 1 and 2. When using a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of DPP4 Working Solution to each well containing the blank control, DPP4 Positive Control, DPP4 Inhibitor Control, and test samples . For a 384-well plate, add 25 µL of DPP4 Working Solution to each well instead.

3. Incubate at room temperature for 10–30 minutes, protected from light.

4. Monitor the fluorescence intensity with a fluorescence microplate reader at Ex/Em = 360/460 nm, Cutoff = 435 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The fluorescence readings in the blank wells (containing only DPP4 Assay buffer) are used as a control. These readings are subtracted from the values obtained from wells with AMC standards, DPP4 positive controls, and test samples. Figure 1 shows the standard curve for AMC. To calculate the AMC concentrations generated from your samples based on this standard curve, we recommend using the Online Linear Regression Calculator, available at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

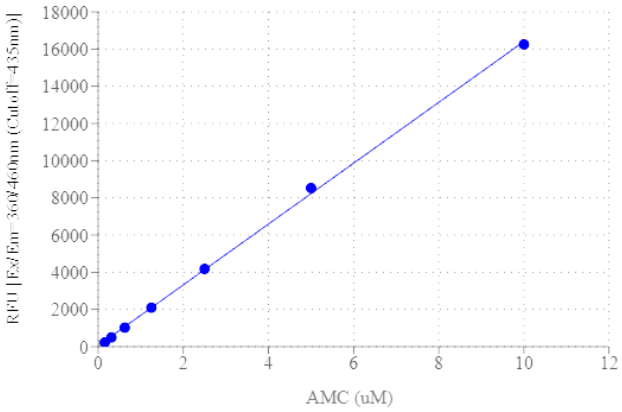

Figure 1. AMC dose response was measured with the Amplite® Fluorimetric DPP4 Activity Assay Kit on a 96-well black microplate after incubation for 30 min at room temperature. The fluorescence intensity was monitored using a Gemini fluorescence microplate reader (Molecular Devices) Ex/Em = 360/460 nm, Cutoff = 435 nm.

Data Analysis

Use the following formula to calculate DPP4 activity:

$$\text{DPP4 Activity} = \Delta \text{ Conc. of AMC (µM)} \div \Delta \text{ T (min)}$$

- Δ Conc. of AMC (µM) = $\text{Con.}_{\text{Final}}$ - $\text{Con.}_{\text{Initial}}$ (µM)
- Δ T (min) = T_{Final} - T_{Initial} (minutes)

Example:

Measure reaction for 30 minutes

DPP4 Con.= 0.5ug/mL

DPP4 Activity (mU/mL) = 0.91uM/10min=0.091mU/mL

DPP4 Activity (U/mg) = 0.182mU/ug=0.182U/mg

DISCLAIMER

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