Amplite® Fluorimetric DPP4 Inhibitor Screening Kit

Catalog number: 11324
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 11324)
Component A: DPP4 Assay Buffer	Freeze (< -15 °C), Minimize light exposure	25 mL
Component B: DPP4 Substrate	Freeze (< -15 °C)	0.35 mL
Component C: DPP4 Enzyme	Freeze (< -15 °C)	20 µL
Component D: DPP4 Inhibitor (Sitagliptin)	Freeze (< -15 °C)	50 µL

OVERVIEW

The Amplite® Fluorimetric DPP4 Inhibitor Screening Kit provides an efficient and direct method for evaluating potential DPP4 inhibitors. DPP4 activity is quantified by the enzymatic cleavage of a specific substrate, resulting in a fluorescent product (Ex/Em = 360/460 nm), which correlates directly with the enzymatic activity present. This kit includes Sitagliptin, a well-characterized DPP4 inhibitor used in diabetes treatment, as a reference control, facilitating the assessment of inhibitor efficacy. The assay is optimized for high-throughput screening applications. Dipeptidyl peptidase-4 (DPP4), also referred to as CD26, ADCP2, or DPP, is a transmembrane glycoprotein within the prolyl oligopeptidase family. DPP4 functions as a serine exopeptidase, cleaving N-terminal X-proline and X-alanine residues from polypeptides. It is implicated in numerous physiological processes, including glucose metabolism via regulation of glucagon-like peptide-1 (GLP-1), immune modulation through its role as a receptor on various immune cells, signal transduction as a transmembrane protein responsive to growth factors and chemokines, and tumor suppression via immune system interactions. Thus, DPP4 inhibitors are critical in the therapeutic management of 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, and the serially diluted DPP4 Inhibitor (Sitagliptin) 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/11324

DPP4 Inhibitor Standard

Add 2.5 µL of DPP4 Inhibitor (Sitagliptin) (Component D) to 497.5 µL of DPP4 Assay Buffer (Component A) to prepare a 50 µM DPP4 Inhibitor Sitagliptin solution (STD7). Then, take 250 µL of STD7 and perform 1:2 serial dilutions in DPP4 Assay Buffer (Component A) to create a series of Sitagliptin 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.

DPP4 Enzyme Working Solution

1. To prepare a 0.5 µg/mL DPP4 Enzyme solution, start with the DPP4 Assay Buffer (Component A). Add 5 µL of the DPP4 Positive Control Stock Solution to 995 µL of the DPP4 Assay Buffer. This mixture will result in a final concentration of 0.5 µg/mL DPP4 Enzyme.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Sitagliptin standards and test samples in a 96-well solid black microplate. (STD = Sitagliptin Standards (STD7-STD1, 50 to 0.0781 µM), BL= Blank Control, TS = Test Samples.)

BL	BL	TS	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	40 µL DPP4 Enzyme Solution + 10 µL Sitagliptin Serial Dilutions (0.078-50 @M)
BL	50 µL	DPP4 Assay buffer
DPP4 Enzyme	50 µL	40 µL DPP4 Enzyme Solution + 10 µL DPP4 Assay buffer
TS	50 µL	40 µL DPP4 Enzyme Solution + 10 µL Test Sample in DPP4 Assay Buffer

1. Prepare the Sitagliptin standards (STD1-7), blank controls (BL), DPP4 Enzyme, 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 Enzyme, 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 from blank wells containing only DPP4 Assay buffer are used as a control. These readings are subtracted from the values of the wells containing the Inhibitor standards, DPP4 Enzyme, and test samples. The standard curve is shown in Figure 1.

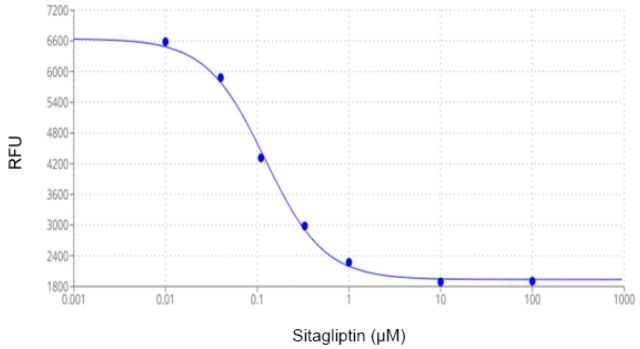

Figure 1. Sitagliptin dose response was measured with the Amplite® Fluorimetric DPP4 Inhibitor Screening Kit on a 96-well black microplate after incubation for 30 minutes at room temperature. The fluorescence intensity was monitored using a Gemini fluorescence microplate reader (Molecular Devices) Ex/Em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

DISCLAIMER

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