

ab133081 – Dipeptidyl peptidase IV (DPP4) Inhibitor Screening Assay Kit

Instructions for Use

For screening DPP4 inhibitors.

View kit datasheet: www.abcam.com/ab133081

(use www.abcam.cn/ab133081 for China, or
www.abcam.co.jp/ab133081 for Japan)

This product is for research use only and is not
intended for diagnostic use.

Table of Contents

1.	Overview	3
2.	Background	4
3.	Components and Storage	5
4.	Pre-Assay Preparation	7
5.	Assay Protocol	9
a.	Data Analysis	14
6.	Troubleshooting	16

1. Overview

ab133081 provides a convenient fluorescence-based method for screening DPP4 inhibitors. The assay uses the fluorogenic substrate, Gly-Pro-Aminomethylcoumarin (AMC), to measure DPP4 activity. Cleavage of the peptide bond by DPP releases the free AMC group, resulting in fluorescence that can be analyzed using an excitation wavelength of 350-360 nm and an emission wavelength of 450-465 nm.

2. Background

Dipeptidyl peptidase IV (DPP4), is also known as CD26 (cluster of differentiation 26 or T-cell activation antigen CD26), or adenosine deaminase complexing protein 2. It is a multifunctional membrane-bound glycoprotein present on the surface of most cell types and is associated with immune regulation, signal transduction, and apoptosis. In humans, DPP4 is ubiquitously expressed in almost all organs and tissues, with the highest expression in kidney, small intestine, and placenta. A soluble form of DPP4 can be found in human serum and seminal fluid and has been characterized as a proteolytic derivative of the membrane-bound form. DPP4 is a serine exopeptidase that cleaves X-proline or X-alanine dipeptides from the N-terminus of polypeptides. There are at least 63 substrates which can bind specifically to DPP4 including growth factors, chemokines, neuropeptides, and vasoactive peptides.

DPP4 inhibitors have emerged as a new class of oral antidiabetic agents. These inhibitors promote glucose homeostasis by inhibiting DPP4, the enzyme responsible for degrading two key glucoregulatory hormones: glucose-dependent insulinitropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). GLP-1 extends the action of insulin while suppressing the release of glucagon. Clinical studies have evaluated the potential for DPP4 inhibition to reduce glucagon levels, delay gastric emptying, and stimulate insulin release. DPP4 inhibitors appear to have excellent therapeutic potential in the management of type 2 diabetes. DPP4

also plays an important role in tumor biology, and is useful as a marker for various cancers, with its levels either on the cell surface or in the serum increased in some neoplasms and decreased in others.

3. Components and Storage

Item	Quantity	Storage
DPP Assay Buffer (10X)	1 vial	-20°C
DPP4 (human recombinant)	2 vials	-80°C
DPP Substrate	1 vial	-20°C
Half Volume 96-Well Solid Plate (white)	1 plate	RT
Sitagliptin Positive Control Inhibitor	1 vial	-20°C
96-Well Cover Sheet	1 cover	RT

Materials Needed But Not Supplied

- A fluorometer with the capacity to measure fluorescence using an excitation wavelength of 350-360 nm and an emission wavelength of 450-465 nm.
- Adjustable pipettes and a repeat pipettor.
- A source of pure water; glass distilled water or HPLC-grade water is acceptable

4. Pre-Assay Preparation

Reagent Preparation

DPP Assay Buffer (10X)

The vial contains 5 ml of buffer. Dilute 3 ml of Assay Buffer concentrate with 27 ml of HPLC-grade water. This final Buffer (20 mM Tris-HCl, pH 8.0, containing 100 mM NaCl, and 1 mM EDTA) should be used in the assay and for diluting reagents. When stored at -20°C, this diluted Buffer is stable for at least six months.

DPP4 (human recombinant)

Each vial contains 120 µl of human recombinant DPP4. Thaw the enzyme on ice; add 480 µl of diluted Assay Buffer to the vial, and vortex. The diluted enzyme is stable for two hours on ice. One vial of enzyme is enough enzyme to assay 60 wells. Use the additional vial if assaying the entire plate.

DPP Substrate

The vial contains 300 µl of 5 mM H-Gly-Pro conjugated to aminomethylcoumarin (AMC). Dilute 120 µl with 2.88 ml of diluted Assay Buffer and vortex. This will be enough Substrate

Solution for 60 wells. Prepare additional Substrate as needed. The Substrate Solution is stable for six hours at room temperature. The addition of 50 μ l to the assay yields a final concentration of 100 μ M Substrate.

NOTE: The K_m value for the peptide is 17.4 μ M. The Substrate concentration in the assay may be reduced by dilution with Assay Buffer at the user's discretion, particularly when assaying for competitive inhibitors (including Sitagliptin Positive Control Inhibitor).

Sitagliptin Positive Control Inhibitor

This vial contains 500 nmol of inhibitor. Resuspend in 500 μ l diluted Assay Buffer to make a 1 mM stock. The addition of 10 μ l to the assay yields a final concentration of 100 μ M inhibitor in the well.

Sample (inhibitors)

Sample (inhibitors) can be dissolved in Assay Buffer or dimethylsulfoxide and should be added to the assay in a final volume of 10 μ l. Ethanol and methanol dramatically reduce enzyme activity and thus they are not recommended for dissolving inhibitors. In the event that the appropriate concentration of inhibitor needed for DPP inhibition is completely unknown, we recommend that several concentrations of the compound be assayed.

5. Assay Protocol

A. Plate Setup

There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as 100% initial activity and three wells designated as background wells. We suggest that each inhibitor sample be assayed in triplicate. A typical layout of samples and compounds to be measured in triplicate is shown in Figure 1 below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BW	BW	BW	7	7	7	15	15	15	23	23	23
B	A	A	A	8	8	8	16	16	16	24	24	24
C	1	1	1	9	9	9	17	17	17	25	25	25
D	2	2	2	10	10	10	18	18	18	26	26	26
E	3	3	3	11	11	11	19	19	19	27	27	27
F	4	4	4	12	12	12	20	20	20	28	28	28
G	5	5	5	13	13	13	21	21	21	29	29	29
H	6	6	6	14	14	14	22	22	22	30	30	30

BW - Background Wells

A - 100% Initial Activity Wells

1-30 – Sample (inhibitor) Wells

Pipetting Hints:

- *It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps to maintain more precise incubation times.*
- *Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times).*

Do not expose the pipette tip to the reagent(s) already in the well.

General Information:

- *The final volume of the assay is 100 μ l in all the wells.*
- *All reagents except the enzyme must be equilibrated to room temperature before beginning the assay.*
- *It is not necessary to use all the wells on the plate at one time.*
- *We recommend assaying samples in triplicate, but it is the user's discretion to do so.*
- *The assay is performed at 37°C.*
- *If the appropriate inhibitor concentration is not known, it may be necessary to assay at several dilutions. A*

dilution series of each inhibitor can be performed to determine IC_{50} values.

- *Thirty inhibitor samples can be assayed in triplicate or forty-five in duplicate.*
- *Monitor the fluorescence with an excitation wavelength of 350-360 nm and an emission wavelength of 450-465 nm.*

B. Performing the Assay

1. 100% Initial Activity Wells - Add 30 μ l of Assay Buffer, 10 μ l of diluted DPP, and 10 μ l of solvent (the same solvent used to dissolve the inhibitor) to three wells.
2. Background Wells - Add 40 μ l of Assay Buffer and 10 μ l of solvent (the same solvent used to dissolve the inhibitor) to three wells.
3. Sitagliptin Positive Control Inhibitor – add 30 μ l of diluted Assay Buffer, 10 μ l of diluted DPP and 10 μ l of the Sitagliptin Positive Control Inhibitor to three wells.
4. Sample (inhibitor) Wells - Add 30 μ l of Assay Buffer, 10 μ l of diluted DPP, and 10 μ l of inhibitor to three wells.
5. Initiate the reactions by adding 50 μ l of diluted Substrate Solution to all the wells.

6. Cover the plate with the plate cover and incubate for 30 minutes at 37°C.
7. Remove the plate cover and read the fluorescence using an excitation wavelength of 350-360 nm and an emission wavelength of 450-465 nm. It may be necessary to adjust the gain setting on the instrument to allow for the measurement of all the samples.

Well	Assay Buffer	DPP	Solvent	Sample (inhibitor)	Substrate Solution
100% Initial Activity	30 µl	10 µl	10 µl	-	50 µl
Background	40 µl	-	10 µl	-	50 µl
Sitagliptin Positive Control Inhibitor	30 µl	10 µl	-	10 µl	50 µl
Sample (inhibitor)	30 µl	10 µl	-	10 µl	50 µl

Table 1. Pipetting summary.

6. Data Analysis

A. Calculations

1. Determine the average fluorescence of 100% Initial Activity, Background, and inhibitor wells.
2. Subtract the fluorescence of the background wells from the fluorescence of the 100% initial activity and inhibitor wells.
3. Determine the percent inhibition for each compound. To do this, subtract each inhibitor sample value from the 100% initial activity sample value. Divide the result by the 100% initial activity value and then multiply by 100 to give the percent inhibition.

$$\% \text{ Inhibition} = \left[\frac{\text{Initial Activity} - \text{Inhibitor}}{\text{Initial Activity}} \right] \times 100$$

4. If multiple concentrations of inhibitor are tested, graph either the Percent Inhibition or Percent Initial Activity as a function of the inhibitor concentration to determine the IC_{50} value (concentration at which there was 50% inhibition). An example of DPP4 inhibition by Sitagliptin is shown in Figure 2.

B. Performance Characteristics

Precision:

When a series of sixteen DPP measurements were performed on the same day, the intra-assay coefficient of variation was 3.9%. When a series of sixteen DPP measurements were performed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 4.1%.

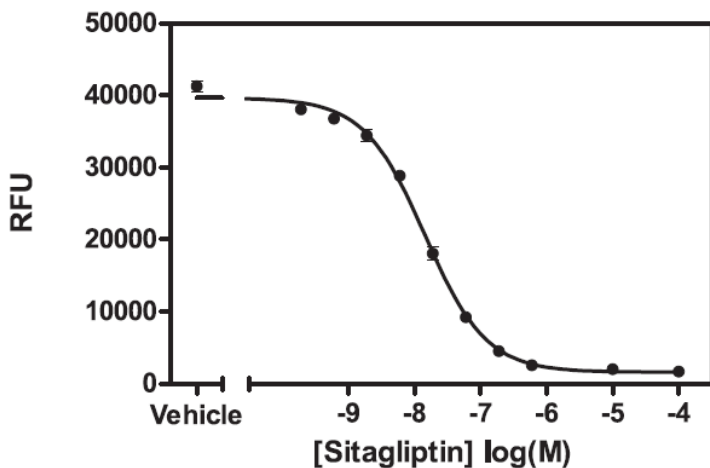


Figure 2. Inhibition of DPP4 by Sitagliptin. Vehicle represents 100 % initial activity.

7. Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells. B. Carefully tap the side of the plate with your finger to remove bubbles.
No fluorescence above background is seen in the Inhibitor wells	Inhibitor concentration is too high and inhibited all of the enzyme activity	Reduce the concentration of the inhibitor and re-assay.
The fluorometer exhibited 'MAX' values for the wells	The GAIN setting is too high.	Reduce the GAIN and re-read.

<p>No inhibition was seen with the inhibitor</p>	<p>A. The inhibitor concentration is not high enough.</p> <p>B. The compound is not an inhibitor of the enzyme.</p>	<p>Increase the inhibitor concentration and re-assay.</p>
---	---	---

UK, EU and ROW

Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria

Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France

Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany

Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain

Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland

Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America

Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada

Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific

Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan

Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp