



# DPP (IV) Inhibitor Screening Assay Kit

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96 assays

Version: 06

Intended for research use only

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## Introduction

### **Background**

Dipeptidyl peptidase IV (DPP (IV)), 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.<sup>1</sup> In humans, DPP (IV) is ubiquitously expressed in almost all organs and tissues, with the highest expression in kidney, small intestine, and placenta.<sup>2</sup> A soluble form of DPP (IV) can be found in human serum and seminal fluid and has been characterized as a proteolytic derivative of the membrane-bound form.<sup>3</sup> DPP (IV) 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 DPP (IV) including growth factors, chemokines, neuropeptides, and vasoactive peptides.<sup>4</sup>

DPP (IV) inhibitors have emerged as a new class of oral antidiabetic agents.<sup>5,6</sup> These inhibitors promote glucose homeostasis by inhibiting DPP (IV), the enzyme responsible for degrading two key glucoregulatory hormones: glucose-dependent insulinotropic 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 DPP (IV) inhibition to reduce glucagon levels, delay gastric emptying, and stimulate insulin release. DPP (IV) inhibitors appear to have excellent therapeutic potential in the management of type 2 diabetes. DPP (IV) 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.<sup>7,8</sup>

### **Principle of the Assay**

DPP (IV) Inhibitor Screening Assay Kit provides a convenient fluorescence-based method for screening DPP (IV) inhibitors. The assay uses the fluorogenic substrate, Gly-Pro-Aminomethylcoumarin (AMC), to measure DPP (IV) 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.

## General Information

### Materials Supplied

List of component

Item	Amount
DPP Assay Buffer (10X)	1 vial
DPP IV (human recombinant)	2 vials
DPP Substrate	1 vial
Sitagliptin Positive Control Inhibitor	1 vial
Half Volume 96-Well Plate (white)	1 plate
96-Well Cover Sheets	1 cover

### Storage Instruction

This kit will perform as specified if stored as stated below and used before the expiration date indicated in the outside of the box.

Item	Storage
DPP Assay Buffer (10X)	-20°C
DPP IV (human recombinant)	-80°C
DPP Substrate	-20°C
Sitagliptin Positive Control Inhibitor	-20°C
Half Volume 96-Well Plate (white)	Room temperature
96-Well Cover Sheets	Room temperature

### Materials Required 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

### Precautions for Use

- ✓ **WARNING:** This product is for research only. Not for human or veterinary diagnostic or therapeutic use.
- ✓ **Precautions:** Please read these instructions carefully before beginning this assay.
- ✓ **Safety Data:** This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet.

✓ 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  $\mu$ L in all the wells.
- Use the diluted Assay Buffer in the assay.
- 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<sub>50</sub> 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

## Assay Protocol

### Reagent Preparation

- ✓ DPP Assay Buffer (10X) - The vial contains 5 mL of 10X 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.
- ✓ DPP IV (human recombinant) - Each vial contains 120 µL of human recombinant DPP (IV). Thaw the enzyme on ice, add 480 µL of diluted Assay Buffer to the vial, and gently mix. The diluted enzyme is stable for two hours on ice. One vial of enzyme is enough 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 µL to the assay yields a final concentration of 100 µM substrate.  
*NOTE: The  $K_m$  value for the peptide is 17.4 µM. 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 µL diluted Assay Buffer to make a 1 mM stock. The addition of 10 µL to the assay yields a final concentration of 100 µM inhibitor in the well.

### Assay Procedure

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 and that you record the contents of each well. A typical layout of samples and compounds to be measured in triplicate is given in Plate Layout

1. 100% Initial Activity Wells - add 30 µL of diluted Assay Buffer, 10 µL of diluted DPP (IV), and 10 µL of solvent (the same solvent used to dissolve the inhibitor) to three wells.
2. Background Wells - add 40 µL of diluted Assay Buffer and 10 µL of solvent (the same solvent used to dissolve the inhibitor) to three wells.
3. Sitagliptin Positive Control Inhibitor – add 30 µL of diluted Assay Buffer, 10 µL of diluted DPP, and 10 µL of the Sitagliptin Positive Control Inhibitor to three wells.
4. Inhibitor Wells - add 30 µL of diluted Assay Buffer, 10 µL of diluted DPP (IV), and 10 µL of inhibitor to three wells.

*Note: Inhibitor can be dissolved in Assay Buffer or dimethylsulfoxide and should be added to the assay in*

a final volume of 10  $\mu\text{L}$ . Ethanol and methanol dramatically reduce enzyme and thus they are not recommended for dissolving inhibitor. In the event that the appropriate concentration of inhibitor needed for DPP (IV) inhibitor is completely unknown, we recommend that several concentrations of the compound be assayed.

5. Initiate the reactions by adding 50  $\mu\text{L}$  of diluted substrate solution to all the wells being used.
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.

*Note: For determination of reaction rates and apparent  $IC_{50}$  values. We recommend reading the sample kinetically, collecting as many time points as possible for the 30 minute assay read time. Determine the initial rate based on the linear portion of the kinetic curve. Calculations can be performed as shown as Table 1 by substituting initial rates for fluorescence.*

Table 1. Pipetting Summary

Well	Assay Buffer	DPP (IV)	Solvent	Inhibitor	Substrate Solution
100% Initial Activity	30 $\mu\text{L}$	10 $\mu\text{L}$	10 $\mu\text{L}$	-	50 $\mu\text{L}$
Background	40 $\mu\text{L}$	-	10 $\mu\text{L}$	-	50 $\mu\text{L}$
Sitagliptin Positive Control Inhibitor	30 $\mu\text{L}$	10 $\mu\text{L}$	-	10 $\mu\text{L}$	50 $\mu\text{L}$
Inhibitor	30 $\mu\text{L}$	10 $\mu\text{L}$	-	10 $\mu\text{L}$	50 $\mu\text{L}$

## Data Analysis

### Calculation of Results

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<sub>50</sub> value (concentration at which there was 50% inhibition). An example of DPP (IV) inhibition by Sitagliptin is shown in Figure 1.

### Performance Characteristics

#### ✓ Z' Factor

Z' Factor is a term used to describe the robustness of an assay<sup>11</sup>, which is calculated using the equation below.

$$Z' = 1 - (3 \sigma_{C+} + 3 \sigma_{C-}) / |\mu_{C+} - \mu_{C-}|$$

Where  $\sigma$ : Standard deviation

$\mu$ : Mean

C+: Positive Control

C-: Negative Control

The theoretical upper limit for Z' factor is 1.0. A robust assay has a Z' factor > 0.5. The Z' factor for this kit was determined to be 0.96.

#### ✓ Sample Data

The data shown here is an example of the data typically produced with this kit; however, your results will not be identical to these. Do not use the data below to directly compare to your samples. Your results could differ substantially.

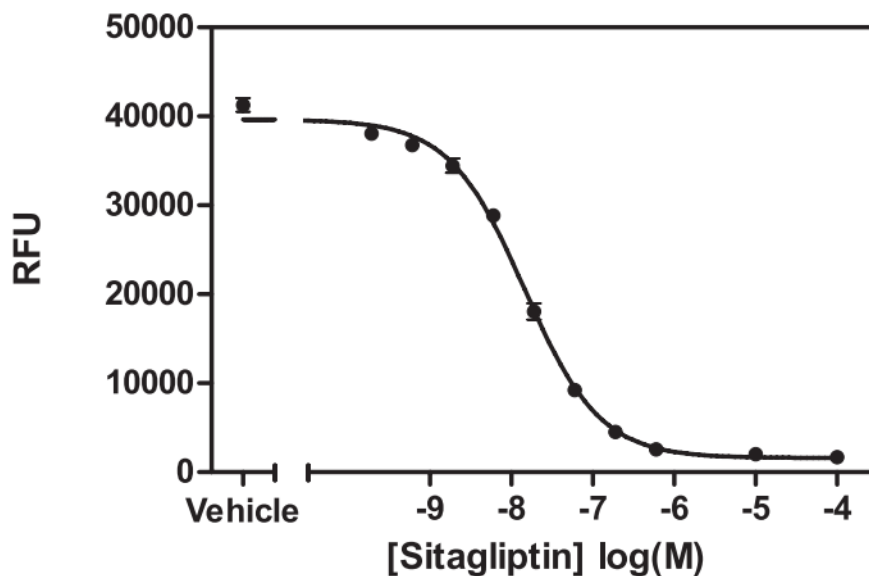


Figure 1. Inhibition of DPP (IV) by Sitagliptin. Vehicle represents 100% initial activity.

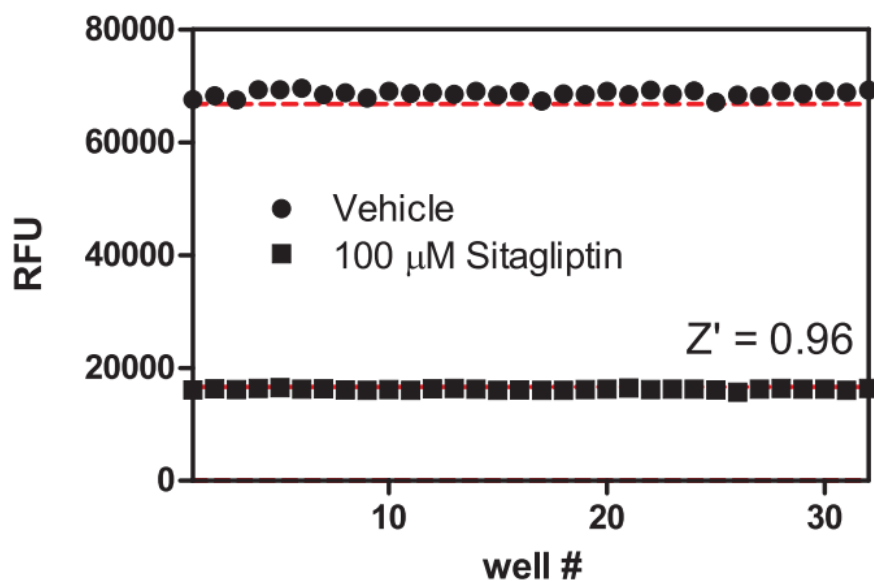


Figure 3. Typical Z' data for the DPP (IV) Inhibitor Screening Assay Kit. Data are shown from wells of both positive and negative controls prepared as described in the protocol. The calculated Z' factor from this experiment was 0.96. The red lines correspond to three standard deviations from the mean of each control value.

## Resources

### Troubleshooting

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

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