DPP4 / DPP (IV) Inhibitor Screening Assay Kit

Cat #: BGT-KAT-100

Size: 96T

Storage: All reagents should be stored as indicated on the component label.

Product information

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. It is a multifunctional membrane-bound glycoprotein present of the surface of most cell types and is associated with immune regulation, signal transduction, and apoptosis. In humans, DPP (IV) is ubiquitously expressed in almost all organs and tissues, with the highest expression in kidney, small intestine, and placenta. 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. DPP (IV) inhibitors have emerged as a new class of oral antidiabetic agents. These inhibitors promote glucose homeostasis by inhibiting DPP (IV), the enzyme responsible for degrading two key glucoregulatory hormones: glucosedependent 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.

Description

DPP4 / 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.

Materials Provided

Item Number	Name	Quantity	Storage
KAT-100-1	DPP Assay Buffer(10X)	1 vial	-20°C
KAT-100-2	DPP(IV)(human recombinant)	2 vials	-80°C
KAT-100-3	DPP Substrate	1 vial	-20°C
KAT-100-4	Sitagliptin Positive Control Inhibitor	1 vial	-20°C
KAT-100-5	Half Volume 96-Well Solid Plate(white)	1 plate	RT
KAT-100-6	96-Well Cover Sheets	1 cover	RT

Materials to be provided by the End-User

1. A fluorometer with the capacity to measure fluorescence using an excitation wavelength of 350-360 nm and an emission wavelength of 450-465 nm

2. Adjustable pipettes and a repeating pipettor

3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

Health Hazard Warnings

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.

2. For Research Use Only.

Reagent Preparation

1. DPP Assay Buffer(10X)

This 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.

2. DPP(IV) (human recombinant)

Each vial contains 120µl of human recombinant DPP(IV). Thaw the enzyme on ice, add 480µl 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 enzyme to

assay 60 wells. Use the additional vial if assaying the entire plate.

3. DPP Substrate

This vial contains 300 ul 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 50µl to the assay yields a final concentration of 100µM Substrate.

NOTE: The K_m value for the peptide is 17.4µM. 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).

4. Sitagliptin Positive Control Inhibitor

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

Plate Set Up

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 on the template sheet. A typical layout of samples and compounds to be measured in triplicate is given 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 - Inhibitor Wells

NOTE:

- The final volume of the assay is 100 µl 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_{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.

Procedure

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: Inhibitors can be dissolved in Assay Buffer or dimethylsulfoxide and should be added to the assay in a final volume of 10 µl. 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 (IV) inhibition is completely unknown, we recommend that several concentrations of the compound be assayed.

5. Initiate the reactions by adding 50 µl 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.

* For determination of reaction rates and apparant IC_{50} values, we recommend reading the samples 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.

Well	Assay Buffer	DPP (IV)	Solvent	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
Inhibitor	30 µl	10 µl	-	10 µl	50 µl

Table. Pipetting summary

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.

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

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).

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.