

# DPP4 Inhibitor Screening Kit (Fluorometric)

(Catalog #K780-100; 100 assays; Store kit at -20°C)

## I. Introduction:

Dipeptidyl peptidase-4 (DPP4) inhibitors have emerged as a new class of oral antidiabetic agents. These inhibitors promote glucose homeostasis by inhibiting degradation of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 by DPP4. Glucagon-like peptide-1 extends the action of insulin while suppressing the release of glucagon. In BioVision's DPP4 Inhibitor Screening Kit, DPP4 cleaves a substrate to release a quenched fluorescent group (Ex/Em = 360/460 nm). In presence of a DPP4 inhibitor, the cleavage will be inhibited. The kit provides a rapid, simple, sensitive, and reliable test, as well as, suitable for high throughput screening of DPP4 inhibitors. Sitagliptin is included as a control inhibitor to compare the efficacy of the test inhibitors.

## II. Kit Contents:

Components	100 assays	Cap Color	Part Number
DPP4 Assay Buffer	25 ml	WM	K780-100-1
DPP4 Substrate	200 µl	Red	K780-100-2
DPP4 Enzyme	100 µl	Green	K780-100-3
DPP4 Inhibitor (Sitagliptin)	50 µl	Blue	K780-100-4

## III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

**DPP4 Enzyme and DPP4 Substrate:** Upon thawing aliquot and store at -20°C, use within 2 months.

**Sitagliptin:** Store at -20°C; use within 2 months.

**DPP4 Assay Buffer:** Store at 4°C or -20°C.

## IV. DPP4 Inhibitor Screening Assay Protocol:

### 1. Enzyme Solution Preparation:

For each well, prepare a total 50 µl DPP4 Enzyme Solution:

- 49 µl Assay Buffer
- 1 µl DPP4 Enzyme

### 2. Screen test inhibitors, inhibitor control and blank control Preparations:

Dissolve test inhibitors into proper solvent. Dilute to 4X the desired test concentration with DPP4 Assay Buffer. For Sitagliptin Inhibitor Control, dilute 1:9 with DPP4 Assay Buffer (**Note:** to compare test inhibitors to Sitagliptin at its IC<sub>50</sub>, dilute Sitagliptin Inhibitor Control 1:99, then use 25 µl). Add 25 µl of test inhibitors, Sitagliptin or DPP4 Assay Buffer into DPP4 enzyme wells as sample screen (S), Inhibitor Control (Sitagliptin), or Enzyme Control (EC, No inhibitor). Mix well, and incubate for 10 minutes at 37°C.

### 3. Substrate Solution Preparation:

- 23 µl DPP4 Assay Buffer
- 2 µl DPP4 Substrate

Mix well and incubate at 37°C.

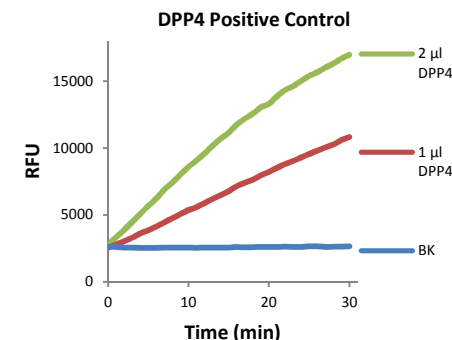
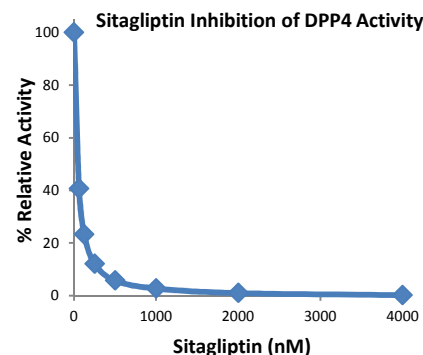
### 4. Measurement:

Measure the fluorescence (Ex/Em = 360/460 nm) in kinetic mode for 15 - 30 min. at 37°C. Protect from light. Choose two time points (T<sub>1</sub> & T<sub>2</sub>) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU<sub>1</sub> and RFU<sub>2</sub>).

### 5. Calculation:

Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU (= RFU<sub>2</sub>- RFU<sub>1</sub>) values by the time ΔT (= T<sub>2</sub>-T<sub>1</sub>). Calculate % Relative Inhibition as follows:

$$\% \text{ Relative Inhibition} = \frac{(\text{Slope of EC} - \text{Slope of S})}{\text{Slope of EC}} \times 100$$



## RELATED PRODUCTS:

- MMP family enzymes, human recombinant
- MMP-1 Inhibitor Screening Kit
- MMP FRET Substrate
- Elastase Inhibitor Screening Kit
- GLP-1 pAb DPPIV, human placenta
- DPPIV Inhibitor, NVP DPP728
- Sitagliptin Phosphate Monohydrate

- MMP family Antibody
- MMP-3 Inhibitor Screening Kit GM6001
- Elastase Inhibitor, SPCK
- DPPIV Inhibitor, K 579
- DPP4 Activity Assay Kit

## GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> <li>• Use of ice-cold assay buffer</li> <li>• Omission of a step in the protocol</li> <li>• Plate read at incorrect wavelength</li> <li>• Use of a different 96-well plate</li> </ul>	<ul style="list-style-type: none"> <li>• Assay buffer must be at room temperature</li> <li>• Refer and follow the data sheet precisely</li> <li>• Check the wavelength in the data sheet and the filter settings of the instrument</li> <li>• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates</li> </ul>
Samples with erratic readings	<ul style="list-style-type: none"> <li>• Use of an incompatible sample type</li> <li>• Samples prepared in a different buffer</li> <li>• Samples were not deproteinized (if indicated in datasheet)</li> <li>• Cell/ tissue samples were not completely homogenized</li> <li>• Samples used after multiple free-thaw cycles</li> <li>• Presence of interfering substance in the sample</li> <li>• Use of old or inappropriately stored samples</li> </ul>	<ul style="list-style-type: none"> <li>• Refer data sheet for details about incompatible samples</li> <li>• Use the assay buffer provided in the kit or refer data sheet for instructions</li> <li>• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated</li> <li>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope</li> <li>• Aliquot and freeze samples if needed to use multiple times</li> <li>• Troubleshoot if needed</li> <li>• Use fresh samples or store at correct temperatures until use</li> </ul>
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> <li>• Improperly thawed components</li> <li>• Use of expired kit or improperly stored reagents</li> <li>• Allowing the reagents to sit for extended times on ice</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw all components completely and mix gently before use</li> <li>• Always check the expiry date and store the components appropriately</li> <li>• Always thaw and prepare fresh reaction mix before use</li> <li>• Refer datasheet &amp; verify correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> </ul>
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> <li>• Use of partially thawed components</li> <li>• Pipetting errors in the standard</li> <li>• Pipetting errors in the reaction mix</li> <li>• Air bubbles formed in well</li> <li>• Standard stock is at an incorrect concentration</li> <li>• Calculation errors</li> <li>• Substituting reagents from older kits/ lots</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw and resuspend all components before preparing the reaction mix</li> <li>• Avoid pipetting small volumes</li> <li>• Prepare a master reaction mix whenever possible</li> <li>• Pipette gently against the wall of the tubes</li> <li>• Always refer the dilutions in the data sheet</li> <li>• Recheck calculations after referring the data sheet</li> <li>• Use fresh components from the same kit</li> </ul>
Unanticipated results	<ul style="list-style-type: none"> <li>• Measured at incorrect wavelength</li> <li>• Samples contain interfering substances</li> <li>• Use of incompatible sample type</li> <li>• Sample readings above/below the linear range</li> </ul>	<ul style="list-style-type: none"> <li>• Check the equipment and the filter setting</li> <li>• Troubleshoot if it interferes with the kit</li> <li>• Refer data sheet to check if sample is compatible with the kit or optimization is needed</li> <li>• Concentrate/ Dilute sample so as to be in the linear range</li> </ul>

**Note:** The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.