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.

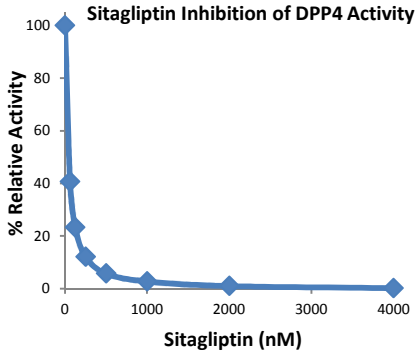
Sitagliptin: Store at -20°C; use within 2 months.

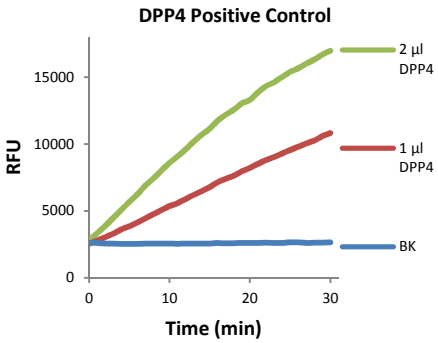
DPP4 Assay Buffer: Store at 4°C or -20°C.

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, dilute1:9 with DPP4 Assay Buffer (**Note**: to compare test inhibitors to Sitagliptin at its IC_{50}, 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:** For each well, prepare a total 25 µl Substrate Solution:
   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_1 & T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 and RFU_2).
5. **Calculation:** Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU (= RFU_2- RFU_1) values by the time ΔT (= T_2-T_1). Calculate % Relative Inhibition as follows:

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





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	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		