DPP4 Activity Fluorometric Assay Kit

(Catalog #K779-100; 100 assays; Store kit at -20°C)

I. Introduction: Dipeptidyl peptidase-4 (DPP4), also known as adenosine deaminase complexing protein 2 or CD26 (cluster of differentiation 26) is a protein that, in humans, is encoded by the DPP4 gene. The substrates of CD26/DPP4 are proline (or alanine) containing peptides and include growth factors, chemokines, neuropeptides, and vasoactive peptides. DPP4 plays a major role in glucose metabolism. It is responsible for the degradation of incretins such as GLP-1 and hence its inhibition by drugs such as Sitagliptin have been used for treatment of diabetes mellitus type 2. DPP4 also appears to work as a suppressor in the development of cancer and tumors. In BioVision's DPP4 Activity Assay Kit, DPP4 cleaves a substrate to release a quenched fluorescent group, AMC (7-Amino-4-Methyl Coumarin), (Ex/Em = 360/460 nm). This assay is rapid, simple, sensitive, and reliable, as well as, suitable for high throughput activity screening of DPP4. This kit detects DPP4 activity as low as 3 µU per well.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
DPP4 Assay Buffer	25 ml	WM	K779-100-1
DPP4 Substrate (H-Gly-Pro-AMC)	200 µl	Red	K779-100-2
DPP4 Positive Control	20 µl	Green	K779-100-3
AMC Standard (1 mM)	100 µl	Yellow	K779-100-4
DPP4 Inhibitor (Sitagliptin)	1 ml	Blue	K779-100-5

III. Storage and Handling:

Store the kit at -20°C, protected from light. Allow DPP4 Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

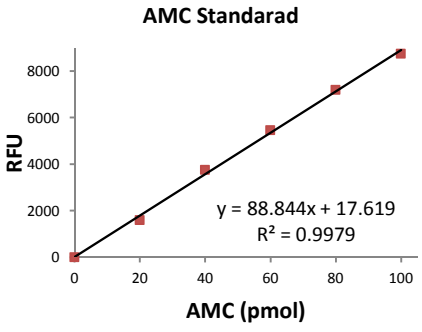
IV. DPPIV Activity Assay Protocol:

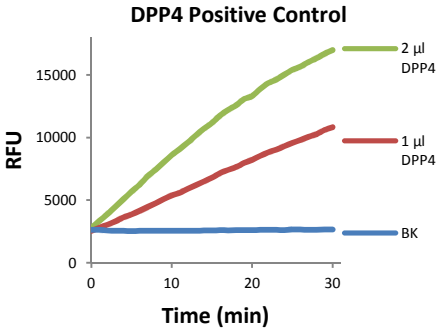
1. **Standard Curve Preparation:** Dilute the AMC Standard 100-fold (10 µl + 990 µl dH_2O) then add 0, 2, 4, 6, 8, 10 µl of the 10 µM AMC (7-Amino-4-Methyl Coumarin) standard into each well individually. Adjust volume to 100 µl/well with DPP4 Assay Buffer to generate 0, 20, 40, 60, 80, 100 pmol/well of AMC standard. Mix and read fluorometrically at Ex/Em = 360/460 nm.
2. **Sample Preparations:**
   Tissues (10 mg) or cells (2 x 10^6) can be homogenized in the 4 volumes of DPP4 Assay Buffer and centrifuged at 13,000 x g for 10 min to remove insoluble material. Serum samples can be directly diluted in the DPP4 Assay Buffer. Prepare duplicate test samples (one for background control-see above) up to 50 µl/well. Adjust to final 50 µl volume into a 96-well plate using DPP4 Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve range. Use 1-2 µl DPP4 as a positive control and adjust volume to 50 µl with DPP4 Assay Buffer.
3. **Background Control:** Add 10 µl DPP4 Assay Buffer to one sample replicate and 10 µl DPP4 Inhibitor to another sample as the sample background control. Mix well and incubate for 10 min at 37 °C.
4. **Reaction Mix:** Prepare reaction mix for each sample:
   38 µl DPP4 Assay Buffer
   2 µl DPP4 Substrate
   Add 40 µl Reaction Mix into each well except the Standard Curve wells. Mix well.
5. **Incubation:** At 37 °C for 30 min (or longer if samples have low DPP4 activity). Read Ex/Em = 360/460 nm R_{S1} and R_{B1} at T_1. Read R_{S2} and R_{B2} again at T_2 after incubating the reaction at 37°C for 30 min (or longer), protected from light. Where S1 and S2 = sample, and B1 and B2 = sample background at times T_1 and T_2, respectively. It is recommended to read kinetically to choose the R_{S1} and R_{S2} at linear range.
6. **Calculation:** The RFU of fluorescence generated by cleavage of substrate by DPP4 is Δ RFU = $(R_{S2} - R_{B2}) - (R_{S1} - R_{B1})$. Plot the AMC Standard Curve, Apply the Δ RFU to the Standard Curve to get B pmol of AMC:

$$\textbf{Activity} = \frac{B}{(T2-T1)\times V} \times \textbf{Sample Dilution Factor} = \textbf{pmol/min/ml} = \textbf{µU/ml}$$

Where: B is the AMC amount from Standard Curve (in pmol).
T_1 is the time of the first reading (R_{1s} and R_{1B}) (in min).
T_2 is the time of the second reading (R_{2S} and R_{2B}) (in min).
V is the sample volume added into the reaction well (in ml).

Unit Definition: One unit is defined as the amount of DPP4 that hydrolyzes the DPP4 Substrate to yield 1.0 µmol of AMC per minute at 37°C.





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
DPP4 Inhibitor Screening Kit
MMP family Antibody
MMP-3 inhibitor screening kit
GM6001
Elastase Inhibitor, SPCK
DPPIV Inhibitor, K 579
Sitagliptin

FOR RESEARCH USE ONLY! Not to be used on humans.

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.		