Product Information

DPP4 Activity Fluorometric Assay Kit

I. Kit Contents:

Components	K2178-100 100 assays	Cap Color	Part Number
DPP4 Assay Buffer	25 ml	WM	K2178-C-1
DPP4 Substrate (H-Gly-Pro-AMC)	200 µl	Red	K2178-C-2
DPP4 Positive Control	20 µl	Green	K2178-C-3
AMC Standard (1 mM)	100 µl	Yellow	A7046
DPP4 Inhibitor (Sitagliptin phosphate monohydrate)	1 ml	Blue	A4036

II. Introduction:

DPP4 (Dipeptidyl peptidase 4, also known as T-celll activation antigen CD26) is a membrane expressed peptidase which exhibits multiple effects throughout the body. It plays an important role in glucose metabolism. It mediates the degradation of incretins (e.g. GLP-1) and as a target for type II diabetes and cancer treatment. In the DPP4 Activity Fluorometric Assay Kit, DPP4 cleaves a substrate to generate a quenched fluorescent group, AMC (7-Amino-4-Methyl Coumarin, Ex/Em = 360/460 nm). This kit provides a fast, selective and robust way for high throughput activity screening of DPP4. The detection limit is 3 µU per well.

III. Storage and Handling:

Store the kit at -20℃, 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℃.

4. Reaction Mix: Prepare reaction mix for each sample:

DPP4 Assay Buffer 38 µl

DPP4 Substrate 2 µl

Add 40 µl Reaction Mix into each well except the Standard Curve wells. Mix well.

5. Incubation:

At 37℃ for 30 min (or longer if samples have low DPP4 activity). Read Ex/Em = 360/460 nm RS1 and RB1 at T1. Read RS2 and RB2 again at T2 after incubating the reaction at 37℃ for 30 min (or longer), protected from light. Where S1 and S2 = sample, and B1 and B2 = sample background at times T1 and T2, respectively. It is recommended to read kinetically to choose the RS1 and RS2 at linear range.

6. Calculation: The RFU of fluorescence generated by cleavage of substrate by DPP4 is ΔRFU = (RS2 – RB2) – (RS1 – RB1). Plot the AMC Standard Curve, Apply the Δ RFU to the Standard Curve to get B pmol of AMC:

Activity =B/ (T2 - T1) × V ×Sample Dilution Factor = pmol/min/ml = µU/ml

Where: B is the AMC amount from Standard Curve (in pmol).

T1 is the time of the first reading (R1s and R1B) (in min).

T2 is the time of the second reading (R2S and R2B) (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℃.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly

Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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.		

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