CREATIVE BIOMART®
Assay Kit

DPP4 Activity Fluorometric Assay Kit

Product Information

Cat.No. Kit-0314

Product Overview

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.

Storage

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.

Size

100 assays

Kit Components

DPP4 Assay Buffer 25 ml
DPP4 Substrate (H-Gly-Pro-AMC) 200 µl
DPP4 Positive Control 20 µl
AMC Standard (1 mM) 100 µl
DPP4 Inhibitor (Sitagliptin) 1 ml

Assay Protocol

1. Standard Curve Preparation: Dilute the AMC Standard 100-fold (10 µl + 990 µl dH2O) 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

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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 RS1 and RB1 at T1. Read RS2 and RB2 again at T2 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 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 Δ RFU = (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°C.