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Product Information

DPP4 Activity Assay Kit

Catalog Number **MAK088**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Dipeptidyl peptidase-4 (DPP4), also known as CD26 and adenosine deaminase complexing protein 2, is a serine exopeptidase that cleaves X-Proline and X-Alanine residues from the N-termini of polypeptides. DPP4 is a transmembrane glycoprotein whose activity regulates the bioactivity of multiple peptides such as growth factors, chemokines, and neuropeptides. DPP4 plays a major role in glucose metabolism via the regulation of glucagon-like peptide-1 and inhibitors of DPP4 are commonly used for the treatment of type 2 diabetes. DPP4 also plays an important role in immune regulation and may play a role in tumor suppression.

The DPP4 Activity Assay kit provides a simple and direct procedure for measuring DPP4 activity in a variety of samples. In this assay, DPP4 cleaves a non-fluorescent substrate, H-Gly-Pro-AMC, to release a fluorescent product, 7-Amino-4-Methyl Coumarin (AMC) (λ_{ex} = 360/λ_{em} = 460 nm). One unit of DPP4 is the amount of enzyme that will hydrolyze the DPP4 substrate to yield 1.0 μmole of AMC per minute at 37 °C.

Components

The kit is sufficient for 100 assays in 96 well plates.

DPP4 Assay Buffer Catalog Number MAK088A	25 mL
DPP4 Substrate, H-Gly-Pro-AMC Catalog Number MAK088B	0.2 mL
DPP4 Positive Control Catalog Number MAK088C	20 μL
AMC Standard, 1 mM Catalog Number MAK088D	0.1 mL
DPP4 Inhibitor, Sitagliptin Catalog Number MAK088E	1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

DPP4 Assay Buffer – Allow buffer to come to room temperature before use.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

AMC Standards for Fluorometric Detection

Dilute 10 μL of the 1 mM AMC Standard solution with 990 μl of water to prepare a 10 μM (10 pmole/μL) standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 10 μM standard solution into a 96 well plate, generating 0 (blank), 20, 40, 60, 80, and 100 pmole/well standards. Add DPP4 Assay Buffer to each well to bring the volume to 100 μL.

Sample Preparation

Tissue (10 mg) or cells (2×10^6) can be homogenized in 4 volumes of ice-cold DPP4 Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material.

Serum samples can be directly added to the wells.

A sample blank is required for each test sample. Prepare a duplicate well for each sample to be used as the sample blank (see Assay Reaction, step 1).

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Bring test samples and sample blanks to a final volume of 50 µL with DPP4 Assay Buffer.

For the positive control, add 1–2 µL of the DPP4 positive control solution to wells and adjust to 50 µL with the DPP4 Assay Buffer.

Assay Reaction

1. Add 10 µL of the DPP4 Assay Buffer to each of the sample wells. Add 10 µL of the DPP4 inhibitor to each of the sample blank wells. Mix well using a horizontal shaker or by pipetting, and incubate for 10 minutes at 37 °C.

2. Set up the Master Reaction Mix according to the scheme in Table 1. 40 µL of the Master Reaction Mix is required for each sample and sample blank well. Do not add the Master Reaction Mix to the Standard Curve wells.

Table 1.
Master Reaction Mix

Reagent	Volume
DPP4 Assay Buffer	38 µL
DPP4 Substrate	2 µL

3. Add 40 µL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Cover the plate and protect from light during the incubation.

4. Incubate the plate at 37 °C. After 5 minutes, take the initial measurement ($T_{initial}$). Measure the fluorescence intensity $(FLU)_{initial}$, λ_{ex} = 360/ λ_{em} = 460 nm).
   Note: It is essential $(FLU)_{initial}$ is in the linear range of the standard curve.

5. Continue to incubate the plate at 37 °C taking measurements (FLU) every 5 minutes. Protect the plate from light during the incubation.

6. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (100 pmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

7. The final measurement $[(FLU)_{final}]$ for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve, see step 5. The time of the penultimate reading is T_{final}.
   Note: It is essential the final measurement falls within the linear range of the standard curve.

Calculations

Correct for the background by subtracting the final measurement [$(FLU)_{final}$] obtained for the 0 (blank) AMC standard from the final measurement [$(FLU)_{final}$] of the standards and samples. Background values can be significant and must be subtracted from all readings. Plot the standard curve.

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in measurement from $T_{initial}$ to T_{final} for the samples.

$$\Delta FLU = (FLU)_{final} - (FLU)_{initial}$$

Also, subtract the Sample Blank Δmeasurement value from the sample Δmeasurement values. Compare the ΔFLU of each sample to the standard curve to determine the amount of AMC released by the DPP4 assay between $T_{initial}$ and T_{final} (B).

The DPP4 activity of a sample may be determined by the following equation:

$$\text{DPP4 Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}$$

B = Amount (pmole) of AMC released between $T_{initial}$ and T_{final}.
Reaction Time = $T_{final} - T_{initial}$ (minutes)
V = sample volume (mL) added to well

DPP4 activity is reported as pmole/min/mL = microunit/mL
One unit of DPP4 is the amount of enzyme that will hydrolyze the DPP4 substrate to yield 1.0 μmole of AMC per minute at 37 °C.

Example:
AMC amount (B) = 58 pmole
First reading ($T_{initial}$) = 5 minute
Second reading (T_{final}) = 35 minutes
Sample volume (V) = 0.05 mL
Sample dilution is 1

DPP4 activity is:

$$\frac{58 \times 1}{(35-5) \times 0.05} = 38.66 \text{ microunits/mL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorometric assays, use clear black plates with clear bottoms
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	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 plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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