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Data Sheet

Fluorogenic DPP4 Assay Kit

Catalog #: w90215
Size: 96 reactions

DESCRIPTION: Dipeptidyl peptidase-4 (DPP4), also known as adenosine deaminase complexing protein 2, is a serine exopeptidase that cleaves X-proline dipeptides from the N-terminus of polypeptides. DPP4 plays a key role in glucose metabolism, immune regulation, signal transduction and apoptosis. The *Fluorogenic DPP4 Assay Kit* is designed to measure DPP4 activity using purified DPP4 for screening and profiling applications. It comes in a convenient 96-well format, with purified DPP4 enzyme, DPP substrate, and DPP assay buffer for 100 enzyme reactions. The key to the *Fluorogenic DPP4 Assay Kit* is the specific, fluorogenic substrate. Using this kit, only one simple step on a microtiter plate is required for DPP4 reactions. The fluorometric substrate is incubated with a sample containing DPP4 enzyme to produce a fluorophore that can then be measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
w90051	DPP4 human recombinant enzyme	1 µg	-80°C	***Avoid freeze/thaw cycles!***
w90311	DPP assay buffer	10 ml	-20°C	
w90316	Fluorogenic DPP substrate 1 in DMSO (0.5 mM)	100 µl	-80°C	
	AMC Fluorescent standard (50 µM)	500 µl	-20°C	
	black, low binding NUNC black microtiter plate	1 plate	Room temp.	

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

REFERENCES:

1. Deacon, C.F., Carr RD, and Holst JJ (2008). *Front. Biosci.* 2008 Jan 1; **13:**1780-94.
2. Langley, A.K., Suffoletta TJ, and Jennings HR (2007). *Pharmacotherapy* **27(8):**1163-80.

WEST BIOSCIENCE

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Immediately prior to assay:

1) Dilute **DPP substrate 1** 0.5 mM stock 5-fold with **DPP assay buffer** to make a 100 µM solution. (Make only sufficient quantity needed for the assay; store remaining 0.5 mM stock solution in aliquots at -20°C.)

2) Dilute **DPP4 enzyme** in **DPP assay buffer** to 0.1 ng/µl (1 ng/reaction)*. Aliquot any remaining enzyme and store undiluted at -80°C. Keep diluted enzyme on ice. Discard any remaining diluted enzyme after use. **Note: Optimal enzyme concentration may vary with the specific activity of the enzyme.*

3) Dilute 25 µl of the **AMC Fluorescent standard** (50 µM stock) 2-fold with **DPP assay buffer** to make a 25 µM solution. Make serial 2-fold dilutions of the fluorescent **AMC standard** in **DPP assay buffer** as follows: 12.5 µM, 6.25 µM, 3.12 µM, 1.56 µM, 0.78 µM, 0.39 µM, 0.20 µM, 0.10 µM. Aliquot the remaining 50 µM **AMC standard** and store undiluted at -20°C. *Note: Protect AMC standard from light*

Step 1:

In duplicate, add the following to the microtiter black plate.

1) Add 80 µl of **DPP Assay Buffer** to each well.

2) Add 5 µl of **DPP Substrate 1** to all wells labeled “Positive Control", “Test Inhibitor”, and “Blank”.

3) Add 5 µl of each diluted **AMC Fluorescent standard** to the wells designated as “AMC Standard Curve”.

4) Add 5 µl of **Inhibitor** solution of each well labeled as “Test Inhibitor”. For the “Positive Control" , “AMC Standard Curve”, and “Blank”, add 5 µl of the same solution without inhibitor (**Inhibitor buffer**).

5) Add 10 µl of **DPP assay buffer** to the wells designated “Blank" and “AMC Standard Curve”.

6) Initiate reaction by adding 10 µl of diluted **DPP4 enzyme** (0.1 ng/µl) to the wells designated “Positive Control” and "Test Inhibitor". Incubate plate at room temperature for 10 min.

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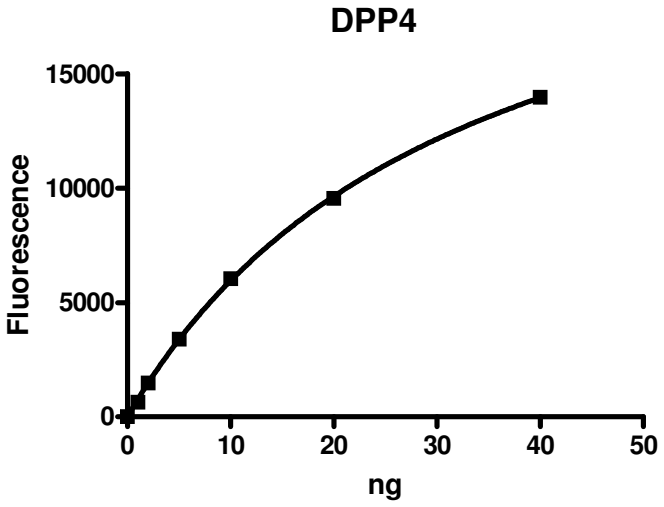
	Enzyme Positive Control	Test Inhibitor	AMC Standard Curve	"Blank" Negative Control
DPP assay buffer	80 µl	80 µl	90 µl	90 µl
DPP substrate 1 (100 µM)	5 µl	5 µl	–	5 µl
AMC standard (0.1 µM – 50 µM)	–	–	5 µl	–
Inhibitor	–	5 µl	–	–
Inhibitor buffer (no inhibitor)	5 µl		5 µl	5 µl
DPP4 (0.1 ng/µl)	10 µl	10 µl	–	–
Total	**100 µl**	**100 µl**	**100 µl**	**100 µl**

Step 2:

Read sample in a microtiter-plate fluorimeter that is capable of excitation at wavelengths ranging from 350-380 nm and detection of emitted light ranging from 440-460 nm. Subtract "Blank" value from all other values.

Example of Assay Results:



DPP4 enzyme activity, measured using the *Fluorogenic DPP4 Assay Kit*, West Bioscience Cat.# w90215. *Note: Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at* sale@westbioscience.com

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