WEST BIOSCIENCE

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Data Sheet

Fluorogenic DPP9 Assay Kit

Catalog #: w90220

DESCRIPTION: Dipeptidyl peptidase-9 (DPP9) is a serine exopeptidase that cleaves X-proline dipeptides from the N-terminus of polypeptides. DPP9 exhibits similar activity to that of DPP4 but unlike DPP4, DPP9 is not membrane bound. DPP9 activity has been linked to several diseases, including type-2 diabetes, obesity and cancer. The *Fluorogenic DPP9 Assay Kit* is designed to measure DPP9 activity using purified DPP9 for screening and profiling applications. It comes in a convenient 96-well format, with purified DPP9 enzyme, DPP substrate, and DPP assay buffer for 100 enzyme reactions. The key to the *Fluorogenic DPP9 Assay Kit* is the fluorogenic substrate. Using this kit, only one simple step on a microtiter plate is required for DPP9 reactions. The fluorogenic substrate is incubated with a sample containing DPP9 enzyme to produce a fluorophore that can then be measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
w90101	DPP9 human recombinant enzyme	1 µg	-80℃	***Avoid freeze/thaw cycles!***
w90311	DPP assay buffer	10 ml	-20℃	
w90316	Fluorogenic DPP substrate 1 in DMSO (0.5 mM)	100 µl	-80℃	
	AMC Fluorescent standard (50 µM)	500 µl	-20℃	
	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. Bjelke,J.R. et al., *Biochem. J.* **396 (2):** 391-399 (2006)
2. Yu, D.M. et al., *FEBS J.* **273 (11):** 2447-2460 (2006)

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ASSAY PROTOCOL:

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℃.)

2) Dilute DPP9 in DPP assay buffer to 1 ng/µl (10 ng/reaction)*. Aliquot any remaining enzyme and store undiluted at -80℃. 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 Fluorescent AMC standard (50 µM stock) 2-fold with DPP buffer to make a 25 µM solution. Make serial 2-fold dilutions of the fluorescent AMC standard in DPP buffer as follows: 12.5 µM, 6.25 µM, 3.12 µM, 1.56 µM, 0.78 µM, 0.39 µM, 0.19 µM, 0.10 µM. Aliquot the remaining 50 µM AMC standard and store undiluted at -20℃.

Step 1:

In duplicate, add the reaction mixtures (below) to the microtiter black plate. Incubate at 22 ºC for 10 min.

	Enzyme Positive Control	**Test Inhibitor**	**AMC Standard Curve**	**Inhibitor Negative Control**	**"Blank" Negative Control**
DPP9 (1 ng/µl)	10 µl	10 µl	–	–	–
DPP substrate 1 (100 µM)	5 µl	5 µl	–	5 µl	–
AMC standard (0.1 µM – 50 µM)	–	–	5 µl	–	–
Inhibitor (in DPP assay buffer)	–	X µl	–	X µl	–
DPP assay buffer	85 µl	85 - X µl	95 µl	95 - X µl	100 µl
Total	**100 µl**	**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.

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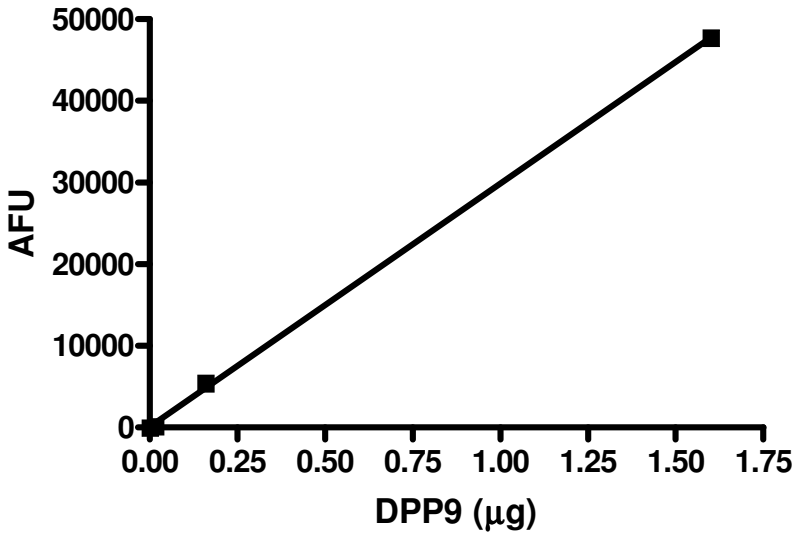
Example of Assay Results:



DPP9 enzyme activity, measured using the *Fluorogenic DPP9 Assay Kit*, West Bioscience Cat.# w90220. *Note: Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at* sale@westbioscience.com.