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Data Sheet

Fluorogenic DPP9 Assay Kit

Catalog #: GWB-67DE80

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:

Ref #	Component	Amount	Storage	
80090	DPP9 human recombinant enzyme	10 µg	-80°C	Avoid freeze/ thaw cycles!
80300	DPP assay buffer	10 ml	-20°C	
80305	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.

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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 μ M solution. (Make only sufficient quantity needed for the assay; store remaining 0.5 mM stock solution in aliquots at -20°C.)
- 2) Dilute DPP9 in DPP assay buffer to 1 ng/ μ l (10 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 μ l of the Fluorescent AMC standard (50 μ M stock) 2-fold with DPP buffer to make a 25 μ M solution. Make serial 2-fold dilutions of the fluorescent AMC standard in DPP buffer as follows: 12.5 μ M, 6.25 μ M, 3.12 μ M, 1.56 μ M, 0.78 μ M, 0.39 μ M, 0.19 μ M, 0.10 μ M. Aliquot the remaining 50 μ M AMC standard and store undiluted at -20°C.

Step 1:

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

	Enzyme Positive Control	Test Inhibitor	AMC Standard Curve	Inhibitor Negative Control	"Blank" Negative Control
DPP9 (1 ng/ μ l)	10 μ l	10 μ l	-	-	-
DPP substrate 1 (100 μ M)	5 μ l	5 μ l	-	5 μ l	-
AMC standard (0.1 μ M – 50 μ M)	-	-	5 μ l	-	-
Inhibitor (in DPP assay buffer)	-	X μ l	-	X μ l	-
DPP assay buffer	85 μ l	85 - X μ l	95 μ l	95 - X μ l	100 μ l
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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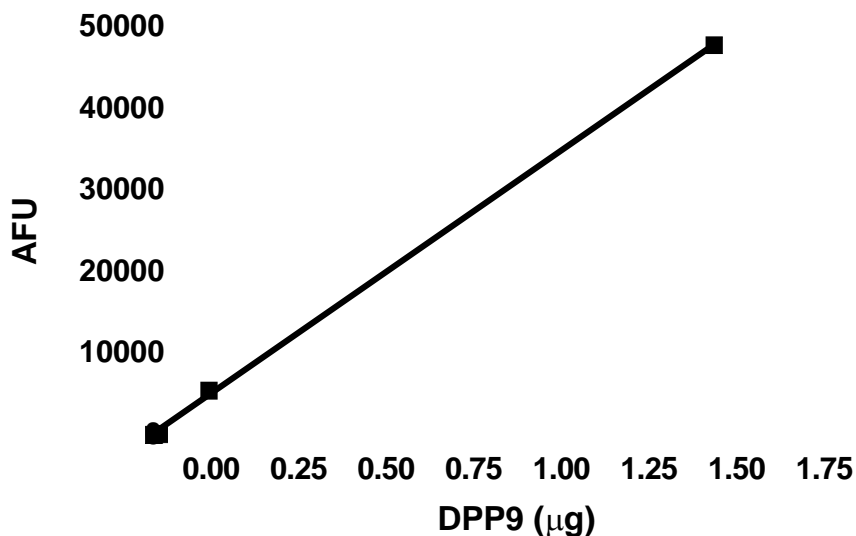
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Example of Assay Results:



DPP9 enzyme activity, measured using the *Fluorogenic DPP8 Assay Kit*, GenWay Biotech, Cat No. **GWB-67DE80**. *Note: Data shown is lot-specific. For lot-specific information, please contact GenWay Biotech at sales@genwaybio.com.*

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