



6405 Mira Mesa Blvd., Suite 100

San Diego, CA 92121

Tel: 1.858.202.1401

Fax: 1.858.481.8694

Email: support@bpsbioscience.com

Data Sheet

Fluorogenic DPP9 Assay Kit

Catalog #: 80209

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	
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	
79685	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)

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°C.)

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- 2) Prepare 10 μ l of inhibitor solution for each well designated "Test Inhibitor." For the Positive Control" and "Blank," prepare 10 μ l of 10% DMSO in DPP assay buffer.
- 3) 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.*
- 4) 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 room temperature for 10 min.

	Enzyme Positive Control	Test Inhibitor	AMC Standard Curve	"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	-	-
AMC standard (0.1 μ M – 50 μ M)	-	-	5 μ l	-
Inhibitor (in DPP assay buffer)	-	10 μ l	-	-
10% DMSO (in DPP assay buffer)	10 μ l	-	-	10 μ l
DPP assay buffer	75 μ l	75 μ l	95 μ l	90 μ l
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.

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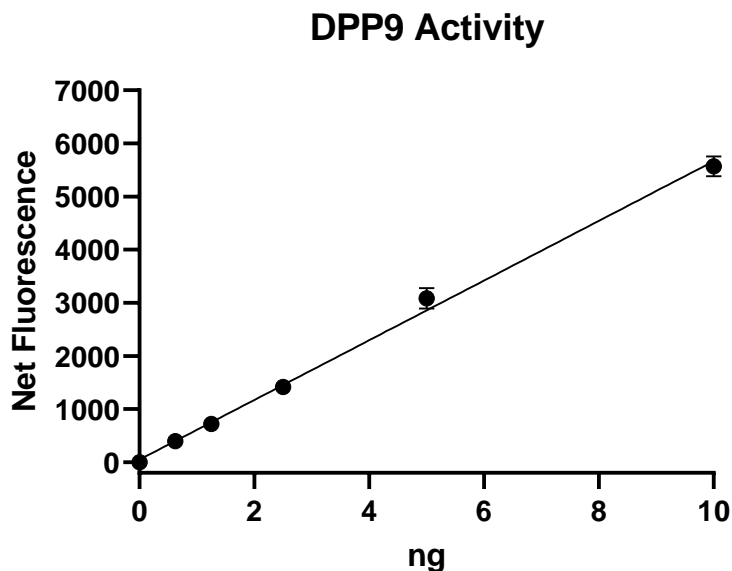
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Example of Assay Results:



DPP9 enzyme activity, measured using the *Fluorogenic DPP9 Assay Kit*, BPS Bioscience Cat. # 80209. *Note: Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.*

RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
DPP9 enzyme	80090	10 µg
DPP assay buffer	80300	20 ml
Fluorogenic DPP substrate 1	80305	100 µl
DPP3 enzyme	80030	10 µg
DPP4 enzyme	80040	10 µg
DPP7 enzyme	80070	10 µg
DPP8 enzyme	80080	10 µg
FAP enzyme	80100	10 µg
POP enzyme	80105	20 µg
Fluorogenic DPP3 assay kit	80203	96 rxns
Fluorogenic DPP4 assay kit	80204	96 rxns
Fluorogenic DPP7 assay kit	80207	96 rxns
Fluorogenic DPP8 assay kit	80208	96 rxns
Fluorogenic FAP assay kit	80210	96 rxns
Fluorogenic POP assay kit	80106	96 rxns
Fluorogenic DPP substrate 2	80332	100 µl

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