



6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet
Fluorogenic DPP4 Assay Kit
Catalog #: 80204
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	
80040	DPP4 human recombinant enzyme	1 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. 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.

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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 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 **DPP4 enzyme** in **DPP assay buffer** to 0.1 ng/ μ l (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 μ l of the **AMC Fluorescent standard** (50 μ M stock) 2-fold with **DPP assay buffer** to make a 25 μ M solution. Make serial 2-fold dilutions of the fluorescent **AMC standard** in **DPP assay 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, 0.20 μ M, 0.10 μ M. Aliquot the remaining 50 μ M **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 μ l of **DPP Assay Buffer** to each well.
- 2) Add 5 μ l of **DPP Substrate 1** to all wells labeled "Positive Control", "Test Inhibitor", and "Blank".
- 3) Add 5 μ l of each diluted **AMC Fluorescent standard** to the wells designated as "AMC Standard Curve".
- 4) Add 5 μ l of **Inhibitor** solution of each well labeled as "Test Inhibitor". For the "Positive Control", "AMC Standard Curve", and "Blank", add 5 μ l of the same solution without inhibitor (**Inhibitor buffer**).
- 5) Add 10 μ l of **DPP assay buffer** to the wells designated "Blank" and "AMC Standard Curve".
- 6) Initiate reaction by adding 10 μ l of diluted **DPP4 enzyme** (0.1 ng/ μ l) to the wells designated "Positive Control" and "Test Inhibitor". Incubate plate at room temperature for 10 min.

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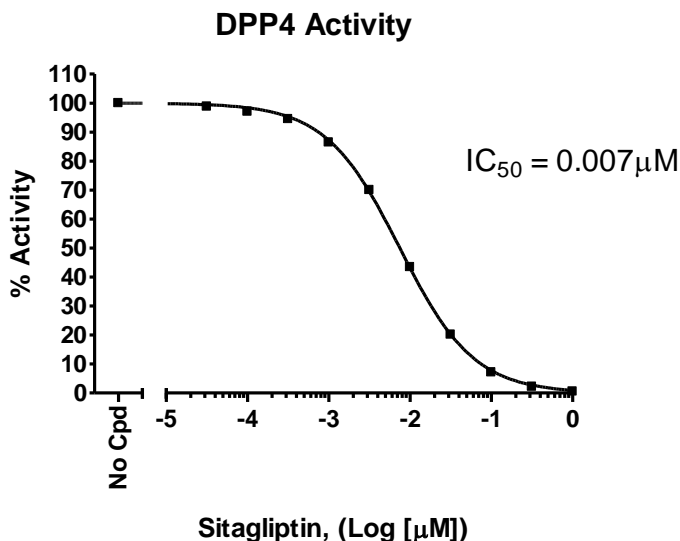
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	Enzyme Positive Control	Test Inhibitor	AMC Standard Curve	“Blank” Negative Control
DPP assay buffer	80 µl	80 µl	90 µl	90 µl
DPP substrate 1 (100 µM)	5 µl	5 µl	-	5 µl
AMC standard (0.1 µM – 50 µM)	-	-	5 µl	-
Inhibitor	-	5 µl	-	-
Inhibitor buffer (no 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*, BPS Bioscience Cat.# 80204. Note: Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

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RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
DPP4 enzyme	80040	10 µg
DPP assay buffer	80300	20 ml
Fluorogenic DPP substrate 1	80305	100 µl
DPP3 enzyme	80030	10 µg
DPP7 enzyme	80070	10 µg
DPP8 enzyme	80080	10 µg
DPP9 enzyme	80090	10 µg
FAP enzyme	80100	10 µg
POP enzyme	80105	20 µg
Fluorogenic DPP3 assay kit	80203	96 rxns
Fluorogenic DPP7 assay kit	80207	96 rxns
Fluorogenic DPP8 assay kit	80208	96 rxns
Fluorogenic DPP9 assay kit	80209	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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