

## Data Sheet

### ***Fluorogenic DPP4 Assay Kit***

**Catalog #: 80204**

**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 or cell extracts containing DPP4. 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:

	Cat. #	Reagent	Amount	Storage
<b>Avoid freeze/thaw cycles!</b>	80040	DPP4 human recombinant enzyme	1 µg	-80 °C
	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.

*Note: The AMC standard is included so the researcher can quantitatively determine the specific activity of the enzyme using the AMC standard as a measure of how much AMC substrate was cleaved to release free AMC.*

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

#### REFERENCES:

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

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## ASSAY PROTOCOL:

### Immediately prior to assay:

- 1) Dilute DPP substrate 1 0.5 mM stock 5-fold with DPP buffer to make a 100  $\mu$ 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 in DPP assay buffer to 0.1 ng/ $\mu$ 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  $\mu$ l of the Fluorescent AMC standard (50  $\mu$ M stock) 2-fold with DPP buffer to make a 25  $\mu$ M solution. Make serial 2-fold dilutions of the fluorescent AMC standard in DPP buffer as follows: 12.5  $\mu$ M, 6.25  $\mu$ M, 3.12  $\mu$ M, 1.56  $\mu$ M, 0.78  $\mu$ M, 0.39  $\mu$ M, 0.19  $\mu$ M, 0.10  $\mu$ M. Aliquot the remaining 50  $\mu$ M AMC standard and store undiluted at -20 °C.

### Step 1:

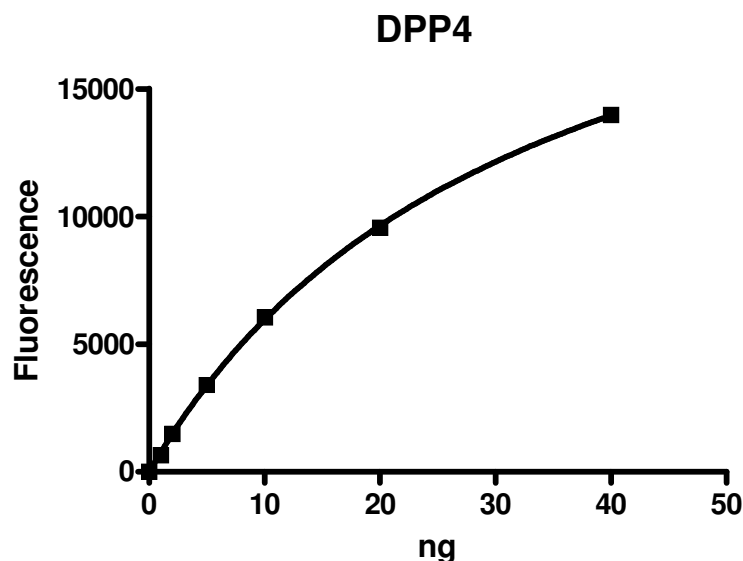
In duplicate, add the reaction mixtures (below) to the microtiter black plate. Incubate at 22 °C for 10 min.

	Enzyme Positive Control	Test Inhibitor	AMC Standard Curve	Inhibitor Negative Control	“Blank” Negative Control
DPP4 (0.1 ng/ $\mu$ l)	10 $\mu$ l	10 $\mu$ l	–	–	–
DPP substrate 1 (100 $\mu$ M)	5 $\mu$ l	5 $\mu$ l	–	5 $\mu$ l	–
AMC standard (0.1 $\mu$ M – 50 $\mu$ M)	–	–	5 $\mu$ l	–	–
Inhibitor (in DPP assay buffer)	–	X $\mu$ l	–	X $\mu$ l	–
DPP assay buffer	85 $\mu$ l	85 - X $\mu$ l	95 $\mu$ l	95 - X $\mu$ l	100 $\mu$ l
<b>Total</b>	<b>100 <math>\mu</math>l</b>	<b>100 <math>\mu</math>l</b>	<b>100 <math>\mu</math>l</b>	<b>100 <math>\mu</math>l</b>	<b>100 <math>\mu</math>l</b>

### 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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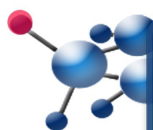
**Example of Assay Results:**

DPP4 enzyme activity, measured using the *Fluorogenic DPP4 Assay Kit*, AMSBIO Cat.# 80204. *Note: Data shown is lot-specific.*

**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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