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Data Sheet

Fluorogenic Prolyl OligoPeptidase (POP) Assay Kit

Catalog # 80106

DESCRIPTION: Prolyl oligopeptidase (POP), also known as prolyl endopeptidase (PREP), is a cytosolic serine peptidase involved in the maturation and degradation of peptide hormones and neuropeptides. The *Fluorogenic Prolyl OligoPeptidase (POP) Assay Kit* is a complete assay system designed to measure activity of the purified POP enzyme. It comes in a convenient 96-well format, with all the reagents necessary for 100 reactions. The *Fluorogenic Prolyl OligoPeptidase (POP) Assay Kit* eliminates the dealing with radioactive materials and chromatography in traditional assays. Purified POP human recombinant enzymeis included in the kit as a positive control. Using this kit, only one simple step, in which the fluorometric substrate is incubated with purified POP, is needed to analyze the POP activity level. The resulting fluorescent product can then be easily measured with a microtiter-plate fluorimeter.

COMPONENTS:

Catalog #	Component	Amount	Storage	
80105	POP human recombinant enzyme	20 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 microtiter plate	1	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.

MATERIALS REQUIRED BUT NOT SUPPLIED: Adjustable micropipettor and sterile tips

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

STABILITY: At least six months from date of receipt when stored as directed.

REFERENCE(S):

1. Myohanen T.T. *et al.* (2007).*Neurochem. Res*. **32 (8):** 1365-1374.
2. Garcia-Horsman J.A. *et al.* (2007). *Scand. J. Gastroenterol*. **42 (5):** 562-571.

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ASSAY PROTOCOL:

Immediately prior to assay:

1) Dilute **Fluorgenic DPP substrate 1 in DMSO (0.5 mM)** stock 10-fold with **DPP assay buffer** to make a 50 µM solution. (Make only sufficient quantity needed for the assay; store remaining 0.5 mM stock solution in aliquots at -20°C.)

2) Dilute **POP human recombinant enzyme** in **DPP assay buffer** to 20 ng/µl (200 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 µl of the **AMC Fluorescent standard (50 µM)** 2-fold with **DPP assay** 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°C.

Step 1:

In duplicate, add the reaction mixtures (below) to the microtiter black plate. Incubate at 22 ºC for 30 min.

	Enzyme Positive Control	Test Inhibitor	AMC Standard Curve	Inhibitor Negative Control	"Blank" Negative Control
POP (20 ng/µl)	10 µl	10 µl	–	–	–
DPP substrate 1 (50 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. Blank value is subtracted from all other values.

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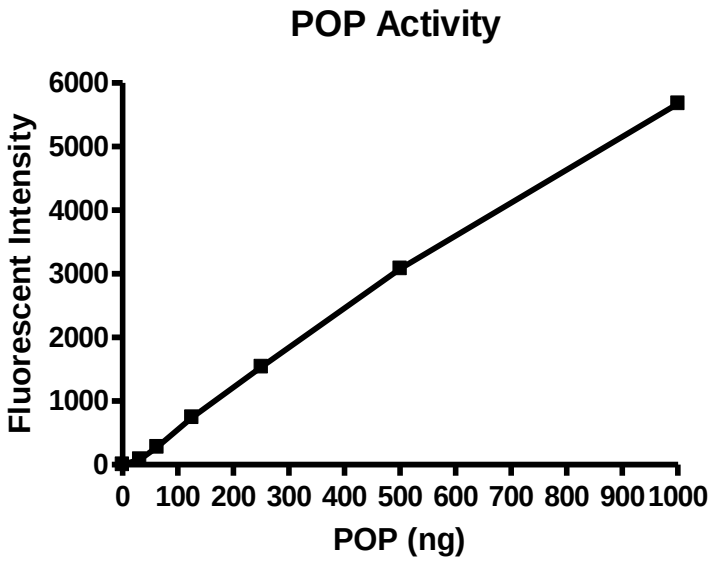
Example of Assay Results:



POP enzyme activity, measured using the *Fluorogenic POP Assay Kit*, BPS Bioscience Cat. # 80106. *Note: Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at* info@bpsbioscience.com.

RELATED PRODUCTS:

Product	Catalog #	Size
POP enzyme	80105	20 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
DPP9 enzyme	80090	10 µg
FAP enzyme	80100	10 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 DPP9 assay kit	80209	96 rxns
Fluorogenic FAP assay kit	80210	96 rxns
Fluorogenic DPP substrate 2	80332	100 µl

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