9891 Irvine Center Dr. Suite 200
Irvine, CA 92618
United States
Tel: 1.800.831.1518
Fax: 1.800.831.1518
Email: sale@westbioscience.com

Data Sheet

Fluorogenic Prolyl OligoPeptidase (POP) Assay Kit

Catalog # w90117

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 POP Assay Kit* eliminates the dealing with radioactive materials and chromatography in traditional assays. Purified human recombinant POP is 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:

	Cat. #	Lot #	Reagent	Amount	Storage
Avoid freeze /thaw cycles !	w90116		POP human recombinant enzyme	20 µg	-80℃
	w90311		DPP assay buffer	10 ml	-20℃
	w90316		Fluorogenic DPP substrate 1 in DMSO (0.5 mM)	100 µl	-80℃
			AMC Fluorescent standard (50 µM)	500 µl	-20℃
			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. 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.

WEST BIOSCIENCE

9891 Irvine Center Dr. Suite 200
Irvine, CA 92618
United States
Tel: 1.800.831.1518
Fax: 1.800.831.1518
Email: sale@westbioscience.com

ASSAY PROTOCOL:

Immediately prior to assay:

1) Dilute DPP substrate 1 0.5 mM stock 10-fold with DPP 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 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 Fluorescent AMC standard (50 µM stock) 2-fold with DPP 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.

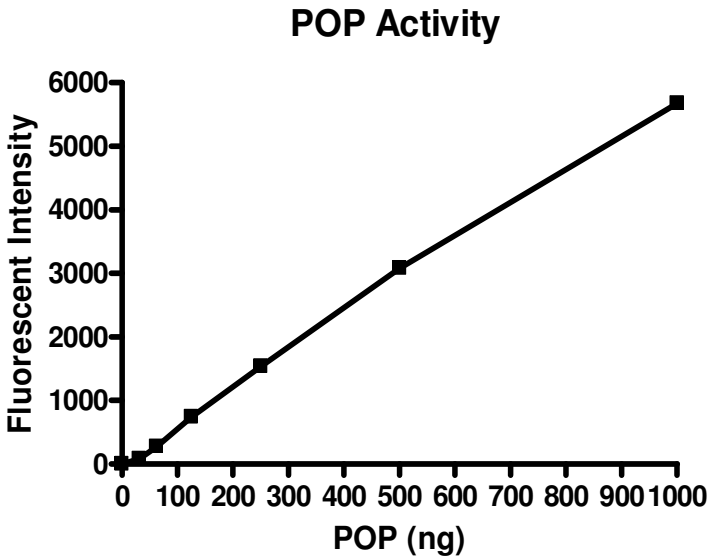
	Enzyme Positive Control	Test Inhibitor	AMC Standard Curve	Inhibitor Negative Control	"Blank" Negative Control
POP (20 ng/µl)	10 µl	10 µl	–	–	–
DPP substrate 1 (50 µM)	5 µl	5 µl	–	5 µl	–
AMC standard (0.1 µM – 50 µM)	–	–	5 µl	–	–
Inhibitor (in DPP assay buffer)	–	X µl	–	X µl	–
DPP assay buffer	85 µl	85 - 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.

9891 Irvine Center Dr. Suite 200
Irvine, CA 92618
United States
Tel: 1.800.831.1518
Fax: 1.800.831.1518
Email: sale@westbioscience.com

Example of Assay Results:



POP enzyme activity, measured using the *Fluorogenic POP Assay Kit*, West Bioscience Cat. # w90117. *Note: Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at* sale@westbioscience.com.