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Data Sheet

Fluorogenic PTP1B (Catalytic Region) Assay Kit

Catalog #79764
Size: 96 reactions

DESCRIPTION: Protein phosphorylation is one of the most important post-translational modification processes. Phosphorylation is reversibly regulated by Protein Kinases (PKs) and Protein Phosphatases (PTPs). PTP1B (PTPN1) is known to catalyze dephosphorylation of insulin receptor kinases and plays a critical role in insulin signaling. The *Fluorogenic PTP1B (Catalytic Region) Assay Kit* is designed to inhibitors of the catalytic region of PTP1B in a homogeneous assay with no time-consuming washing steps. The PTP1B assay kit comes in a convenient 96-well format, with purified PTP1B enzyme, fluorogenic substrate, and PTP assay buffer for 100 enzyme reactions. Note: To identify inhibitors of the regulatory region of PTP1B, please use our *Fluorogenic PTP1B (Full Length) Assay Kit,* #79766)

COMPONENTS:

Catalog #	Component	Amount	Storage	
30010	Recombinant Human PTP1B (1-321)	≥1 µg	-80°C	***Avoid freeze/ thaw cycles!***
	0.5 mM PTP Substrate	50 µl	-80°C	
79716	5X PTP Assay Buffer	20 ml	-20°C	
	0.5 M DTT	20 µl	-20°C	
79685	Black, low binding black microtiter plate	1	Room Temperature	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of reading exc/em=360nm/460nm

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

STABILITY: One year from date of receipt when stored as directed.

REFERENCE(S):
Gee, K.R., *et al., Anal Biochem*, 1999 Aug 15; **273(1):**41-8.
Brown-Shimer, S., *et al.,* 1992 Jan 15; **52(2):**478-82.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Prepare **1X PTP Assay Buffer** with 1 mM DTT from **5X PTP Assay Buffer**. For example, add 200 µl **5X PTP Assay Buffer** and 2 µl 0.5M DTT to 798 µl distilled H_2O to make 1 ml **1X PTP Assay Buffer**.

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2) Prepare the master mixture: N wells × (24.5 µl **1X PTP Assay Buffer** (with DTT) + 0.5 µl 0.5 mM PTP Substrate).

3) Add 25 µl of master mixture to each well (Final concentration of the PTP substrate in a 50 µl reaction is 5 µM).

4) Prepare the inhibitor solution.

   If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound in DMSO than the highest concentration you want to test in the assay. Then make a 10 fold dilution in 1X assay buffer (at this step the compound concentration is 10-fold higher than the final concentration). If you want to run an IC50 or test lower concentrations of the compound, make a series of further dilutions using 1X assay buffer containing 10% DMSO, so the final concentration of DMSO will be 1% in all samples.

   If the inhibitor compound is dissolved in water, make a solution of the compound in 1X assay buffer that is 10-fold higher than the final assay concentration.

5) Add 5 µl of the inhibitor solution to the well designed as "Test Sample". Add 5 µl of the inhibitor buffer (without inhibitor) to the wells designed as "Blank", and "Positive Control".

Component	Positive Control	Test Sample	Blank
1X assay buffer with DTT	24.5 µl	24.5 µl	44.5 µl
Substrate	0.5 µl	0.5 µl	0.5 µl
Test Inhibitor	–	5 µl	–
Inhibitor Buffer (no inhibitor)*	5 µl	–	5 µl
PTP1B (2 pg/µl)	20 µl	20 µl	–
Total	**50 µl**	**50 µl**	**50 µl**

***** Inhibitor buffer typically represents 1x PTP assay buffer with proper concentration of DMSO.

6) Thaw **Recombinant Human PTP1B (1-321)** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **Recombinant Human PTP1B (1-321)** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: PTP1B enzyme is sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) Dilute **Recombinant Human PTP1B (1-321)** in **1X PTP Assay Buffer** at 2 pg/µl (40 pg per reaction).

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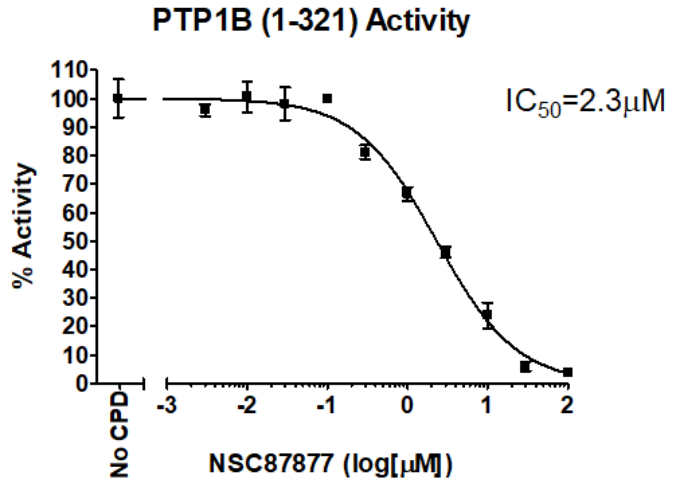
8) Add 20 µl diluted **Recombinant Human PTP1B (1-321)** solution to wells designated "Positive Control" and "Test Sample". Add 20 µl 1X assay buffer to "Blank" wells.

9) Incubate at room temperature for 30 minutes. Measure the fluorescence intensity in a microtiter plate-reading fluorimeter capable of excitation at a wavelength 360 nm and detection of emission at a wavelength 460 nm. You can also measure the fluorescence intensity kinetically. "Blank" value is subtracted from all other values.

Example of Assay Results:



PTP1B enzyme activity, measured using the *Fluorogenic PTP1B (Catalytic Region) Assay Kit (BPS Bioscience #79764).* Fluorescence intensity was measured using a Tecan fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

Related Products

Product	Cat. #	Size
Human PTP1B (1-321), GST-tag	30010	20 µg
PTP1B (1-321) Colorimetric Assay Kit	30019	96 rxns.
Mouse PTP1B (1-321), GST-tag	30012	20 µg
Rat PTP1B (1-321), GST-tag	30011	20 µg
PTP1B (PTPN1) full length, GST-tag	30009	20 µg
10x PTP1B Colorimetric Substrate	79693	5 ml