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Data Sheet

PTP1B (Catalytic Domain) Colorimetric Assay Kit

Catalog #30019

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 *PTP1B (Catalytic Domain) Colorimetric Assay Kit* is designed to measure PTP1B activity for screening and profiling applications, 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, colorimetric substrate, and PTP assay buffer for 100 enzyme reactions. The *PTP1B (Catalytic Domain) Colorimetric Assay Kit* is optimized for both continuous and end-point assay formats.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
30010	PTP1B (1-321), GST-tag*	10 µg	-80°C	***Avoid freeze/ thaw cycles!***
79716	5x PTP assay buffer	3 ml	-20°C	
79693	10x PTP1B Colorimetric Substrate	1 ml	-20°C	
79963	Transparent 96-well plate	1	Room Temp.	

**PTP1B concentration is lot-specific and will be indicated on the tube containing the enzyme.*

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
DTT (or other reducing agents such as β-mercaptoethanol or TCEP)
UV/Vis spectrophotometer microplate reader capable of reading absorbance at 410-420 nm
Adjustable micropipettor and sterile tips
Rotating or rocker platform

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

STABILITY: Up to 1 year when stored as recommended.

REFERENCE: Barrett, W.C., *et al., Biochem.* 1999; **38**: 6699-6705

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

Continuous Assay:

1) Thaw **5x PTP assay buffer** and **10x PTP1B colorimetric substrate** on ice.

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2) Prepare the master mixture: N wells x (16 µl **5x PTP assay buffer** + 10 µl **10x PTP1B Colorimetric Substrate** + 2 µl DTT* (100 mM) + 42 µl water). Add 70 µl to every well. *100 mM β-mercaptoethanol or 50 mM TCEP can be used instead.

	Positive Control	Test Inhibitor	Blank
5x PTP assay buffer	16 µl	16 µl	16 µl
10x PTP1B Colorimetric Substrate	10 µl	10 µl	10 µl
DTT (100 mM)	2 µl	2 µl	2 µl
Water	42 µl	42 µl	42 µl
Test Inhibitor	–	10 µl	–
Inhibitor Buffer (no inhibitor)	10 µl	–	10 µl
1x PTP assay buffer	–	–	20 µl
PTP1B (~ 5 ng/µl)	20 µl	20 µl	–
Total	100 µl	100 µl	100 µl

3) Add 10 µl of Inhibitor solution of each well labeled as “Test Inhibitor”. For the “Positive Control" and “Blank”, add 10 µl of the same solution without inhibitor (Inhibitor buffer). *Note: The PTP1B (Catalytic Domain) Colorimetric Assay Kit is compatible with up to 1% final DMSO concentration. We recommend preparing the inhibitor in 10% DMSO aqueous solution and using 10 µl per reaction.*

4) Prepare **1x PTP assay buffer** by diluting 1 part **5x PTP assay buffer** with 4 parts distilled water. Add 20 µl of **1x PTP assay buffer** to the wells designated as “Blank”.

5) Thaw **PTP1B** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **PTP1B** required for the assay and dilute enzyme to ~ 5 ng/µl with **1x PTP assay buffer**. Aliquot remaining **PTP1B** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **PTP1B** enzyme is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Set the microplate reader to read absorbance at 415 nm for 1 h at room temperature. Two minute intervals between readings is recommended.

7) Initiate reaction by adding 20 µl of diluted **PTP1B** enzyme to the wells designated “Positive Control” and "Test Inhibitor Control". Incubate at room temperature for 1 hour.

8) Read the plate continuously for 1 hour and use the slope of absorbance increase to calculate the activity.

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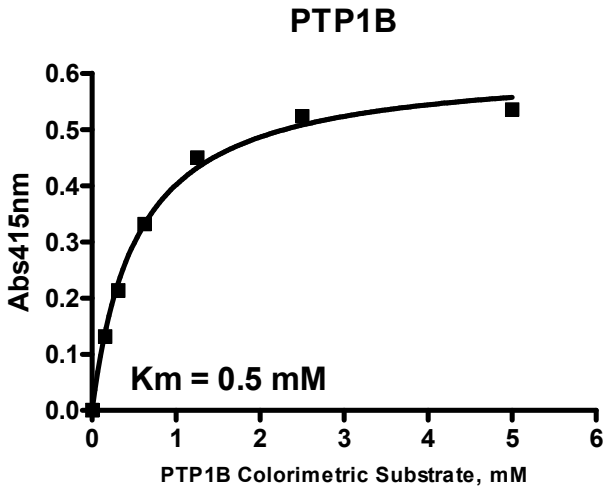
End-point Assay:

1) Follow steps 1 through 5 for the Continuous Assay (above)
2) Set the microplate reader to read absorbance at 415 nm.
3) Read the plate at 415 nm and record the initial absorbance.
4) Initiate reaction by adding 20 µl of diluted **PTP1B** enzyme to the wells designated “Positive Control” and "Test Inhibitor Control". Incubate at room temperature for 1 hour.
5) Read the plate at 415 nm and record the final absorbance.
6) Use the difference between final and initial absorbance to calculate the activity.

Example of Assay Results:



RELATED PRODUCTS:

Product Name	Catalog #	Size
Human PTP1B (PTPN1) full length, GST-tag	30009	20 µg
Human PTP1B (PTPN1) 1-321, GST-tag	30010	20 µg
Rat PTP1B (PTPN1) 1-321, GST-tag	30011	20 µg
Mouse PTP1B (PTPN1) 1-321, GST-tag	30012	20 µg

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Colorimetric signal of positive control reaction is weak	Enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme. Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity.
	Colorimetric substrate was not incubated long enough	Increase the amount of time that the assay is incubated before reading absorbance. Avoid azides.
Colorimetric signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
	Signal is out of range of detection (too high)	Decrease the amount of time that the assay is incubated before reading absorbance.
Background (signal to noise ratio) is high	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of enzyme to create a standard curve.