

PTP1B Inhibitor Screening Assay Kit ab139465

2 References 6 Images

Overview

Product name	PTP1B Inhibitor Screening Assay Kit
Detection method	Colorimetric
Sample type	Purified protein, Inhibitor compounds
Assay type	Enzyme activity
Product overview	<p>Abcam PTP1B Inhibitor Screening Assay Kit (ab139465) is a colorimetric, non-radioactive assay designed to measure the phosphatase activity of purified PTP1B. This 96-well assay is useful for screening inhibitors and modulators of PTP1B activity.</p> <p>The kit includes human, recombinant PTP1B (residues 1-322; MW=37.4 kDa), expressed in <i>E. coli</i> as a positive control. The PTP1B inhibitor suramin is including as control for inhibitor detection.</p> <p>The detection of free-phosphate released is based on the classic Malachite green assay and offers the advantages of convenient, one-step detection and excellent sensitivity, without radioactivity.</p>

Notes	<p>Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.</p> <p>It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.</p>
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Platform	Microplate reader
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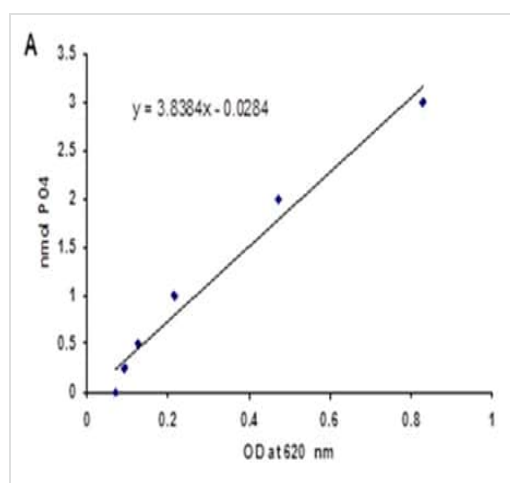
Properties

Components	1 x 96 tests
96-well Clear Microplate (1/2 Volume)	1 unit
Phosphate Standard	1 x 0.5ml
PTP1B Assay Buffer	1 x 20ml
PTP1B Enzyme (Human, Recombinant)	1 x 5µg

Components	1 x 96 tests
PTP1B Inhibitor	1 x 10mg
PTP1B Substrate	1 x 1mg
Red Assay Reagent	1 x 5ml

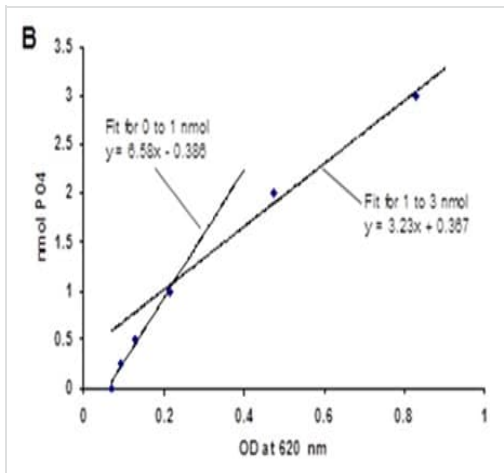
Function	May play an important role in CKII- and p60c-src-induced signal transduction cascades.
Sequence similarities	Belongs to the protein-tyrosine phosphatase family. Non-receptor class 1 subfamily. Contains 1 tyrosine-protein phosphatase domain.
Post-translational modifications	Oxidized on Cys-215; the Cys-SOH formed in response to redox signaling reacts with the alpha-amido of the following residue to form a 4-amino-3-isothiazolidinone serine cross-link, triggering a conformational change that inhibits substrate binding and activity. The active site can be restored by reduction.
Cellular localization	Endoplasmic reticulum membrane.

Images



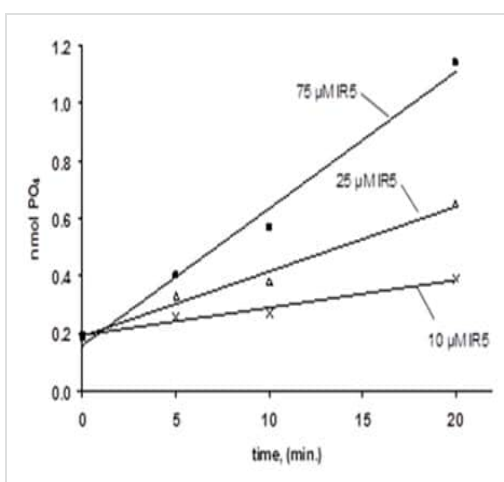
Phosphate Standard Curve.

Dilutions of phosphate standard and a buffer blank were prepared. The 100 μ L samples were mixed with 25 μ L Red Assay Reagent and incubated at 30°C, 20 min to develop color. OD620nm was read on a microplate-reading spectrophotometer. Least-squares fit to the entire set of phosphate amounts, from 0 to 3 nmol.



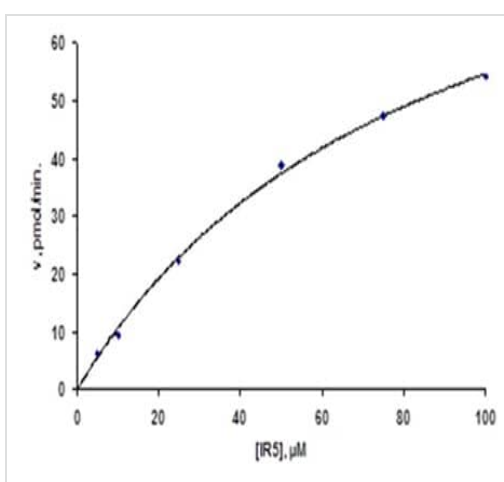
Phosphate Standard Curve.

Dilutions of phosphate standard and a buffer blank were prepared. The 100 μ L samples were mixed with 25 μ L Red Assay Reagent and incubated at 30°C, 20 min to develop color. OD_{620nm} was read on a microplate-reading spectrophotometer. A more accurate correlation of OD₆₂₀ to phosphate is obtained by separate fits of the data from 0 to 1 nmol and 1 to 3 nmol.



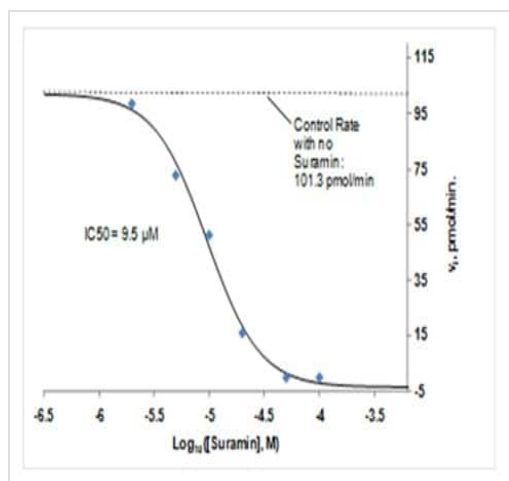
Time Courses of PTP1B Phosphate Release from the PTP1B Substrate.

2X PTP1B Substrate solutions (150, 50 and 20 μ M) and 2X PTP1B Enzyme solutions (2 ng/well) were prepared and incubations at 30°C were performed. Reactions were then terminated by addition of 25 μ L of Red Assay Reagent and OD₆₂₀ read. OD₆₂₀ readings were converted to nmol of PO₄ with a phosphate standard curve. Each point represents the mean of two determinations.



Dependence of PTP1B Kinetics on PTP1B Substrate (IR5) Concentration.

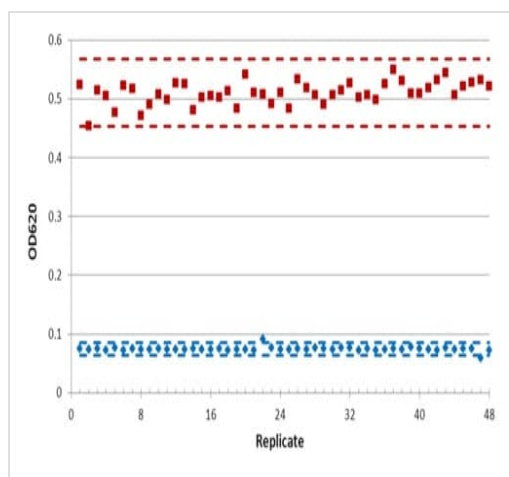
Initial rates of IR5 (PTP1B Substrate) dephosphorylation by 2 ng of PTP1B were determined at 30°C and the indicated concentrations from 20 min. Time course plots (See Fig. 2 and Data Analysis). The line is a non-linear least squares fit to the Michaelis-Menten equation. The K_m for IR5 (PTP1B Substrate) was 85 μ M and the V_{max} was 101 pmol/min.



Inhibition of PTP1B by Suramin (PTP1B Inhibitor).

Phosphate release from 75 μM PTP1B Substrate by 2.5 ng PTP1B (30°C) was measured at 10 min. Intervals for 30 min at suramin concentrations of 0 to 100 μM . Data were converted from OD620 to pmol of phosphate by means of standard curves (see Fig. 1B) and initial rates determined from best fits to the linear parts of plots of pmol phosphate vs. time (0-20 min. for 0 and 2 μM suramin, 0-30 min. for all other concentrations). The dose-response curve was derived from a least squares fit to the 4-parameter Hill-Slope model:

(see Protocol Figure 4 legend text). The fitted parameter values were: top = 101.9 pmol/min; bottom = -3.5 pmol/min; IC50 = 9.5 μM ; slope = 1.8.



Z-Factor Analysis

PTP1B (2.5 ng) (red squares) or buffer (blue diamonds) was incubated for 20 minutes at 30°C with 75 μM PTP1B Substrate. Reactions were then terminated by addition of 25 μL of Red Assay Reagent and OD620 read. The Z' factor for this assay was 0.84, (Z-factor = $1 - ((3\text{SDpositive} + 3\text{SDnegative}) / (\text{mean positive} - \text{mean negative}))$). Dashed lines indicate the 3*Standard deviation range.

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