

ProFluor™ Tyrosine Phosphatase Assay

Description

The ProFluor™ Tyrosine Phosphatase Assay measures the activity of purified tyrosine protein phosphatases in a multiwell plate format and involves “add, mix, and read” steps only. The assay works with such tyrosine protein phosphatase as PTP-1B, CD45 PTPase, YOP-51, PAR PTPase, and T-cell PTPase (Figures 2 and 3). The assay begins with a standard phosphatase reaction performed with the provided phosphorylated bisamide rhodamine 110 peptide substrate (PTPase R110 Substrate) and Control AMC Substrate that serves as a control for compounds that may inhibit the assay (Figure 1). In this configuration, both the PTPase R110 Substrate and Control AMC Substrate are nonfluorescent. Following the phosphatase reaction, the addition of a protease solution simultaneously stops the phosphatase reaction and completely digests the dephosphorylated PTPase R110 Substrate and the Control AMC substrate, producing highly fluorescent rhodamine 110 and AMC. Phosphorylated PTPase R110 Substrate, however, is resistant to protease digestion and remains nonfluorescent. Thus, the R110 fluorescence intensity measured in the assay directly correlates with phosphatase activity (Figure 2), while the AMC fluorescence intensity is a monitor of protease activity. The assay produces excellent Z' values (> 0.7) in either 96- or 384-well plate formats and produces IC_{50} values for known phosphatase inhibitors that agree closely with published values (1,2).

A compound that only inhibits the phosphatase will decrease the R110 fluorescent signal but has no effect on the AMC signal. A protease inhibitor will decrease both AMC and R110 signals. To distinguish protease inhibitors from phosphatase inhibitors, the AMC fluorescence signal should be examined carefully where a decrease in R110 fluorescence was observed.

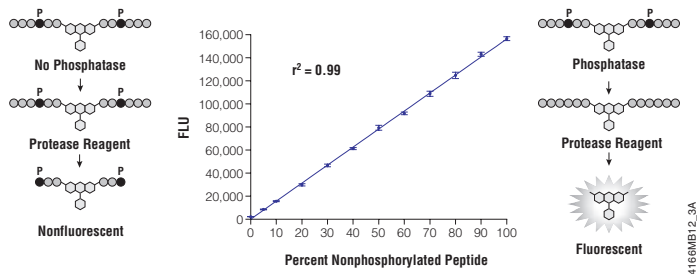


Figure 1. Effect of phosphopeptide content on fluorescence intensity in the ProFluor™ Phosphatase Assays. Schematic demonstrates the principle behind the ProFluor™ Tyrosine Phosphatase Assay where the unphosphorylated peptide substrate is readily digested by a protease reagent leading to the release of highly fluorescent rhodamine 110. Phosphorylated substrate (indicated by dark circles), however, is resistant to digestion by the protease and does not contribute to the fluorescent signal. The graph shows fluorescence intensity as a function of percent unphosphorylated substrate following a protease digestion (Serine/Threonine Peptide Substrate data shown) to mimic a phosphatase titration. FLU = Fluorescence Light Units.

Features

- **Screen More Compounds:** The micromolar concentration of rhodamine 110-conjugated substrate used in the assay produces fluorescent signals much higher than the intrinsic fluorescence of most problematic test compounds.
- **Homogeneous and Nonradioactive:** Simple “add, mix and read” format with none of the safety and disposal costs associated with radiometric assays.
- **Low False-Positive Rate:** The Protease Reagent in the ProFluor Tyrosine Phosphatase Assay was tested against the Library of Pharmacologically Active Compounds (LOPAC- Sigma-RBI). None of the 640 LOPAC library compounds interfered with the Protease Reagent (data not shown). To further ensure against picking up false-positives, a control peptide (AAF-AMC) is included that is used to monitor protease activity.
- **Sensitive:** Requires just nanogram quantities of enzyme per well.
- **Highly Predictive Results:** Signal-to-background ratios of $>50:1$ and low well-to-well variability result in Z' values of 0.7 or greater, leading to highly predictive results (Figure 4).
- **Accurate IC_{50} values:** IC_{50} values obtained using the ProFluor™ Tyrosine Phosphatase Assay for all measured PTPase inhibitors agree closely with published values (Figure 5).

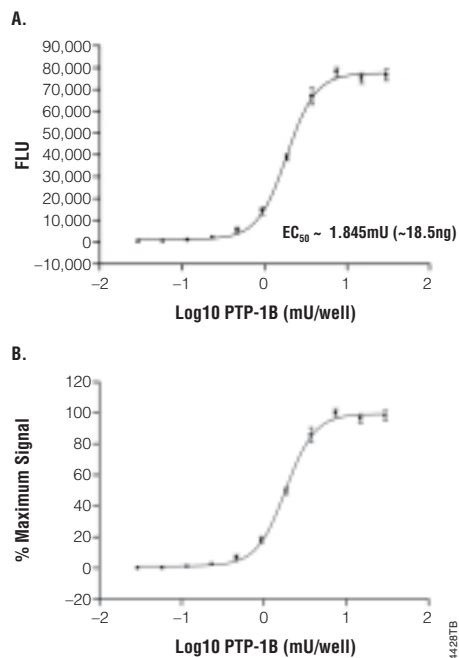


Figure 2. PTPase activity correlates with R110 fluorescence output. Results of titration curves that were performed in solid-black, flat-bottom 96-well plates. **Panel A** and **B** show the results of a PTP-1B titration (Calbiochem Cat. # 539735). **Panel A** is the data collected (actual R110 FLU units) from the plate. Data points are the average of 8 determinations, and error bars are \pm S.D. Curve fitting was performed using GraphPad Prism® 4.0 sigmoidal dose-response (variable slope) software. The r^2 value is 0.99, EC_{50} is 1.85mU/well (~18.5ng), and the maximum dynamic range in the assay is ~1,000-fold. Normalizing the data allows for a quick determination of the amount of phosphatase required for the desired percent conversion (85–90% recommended for Z' and screening experiments) (**Panel B**).

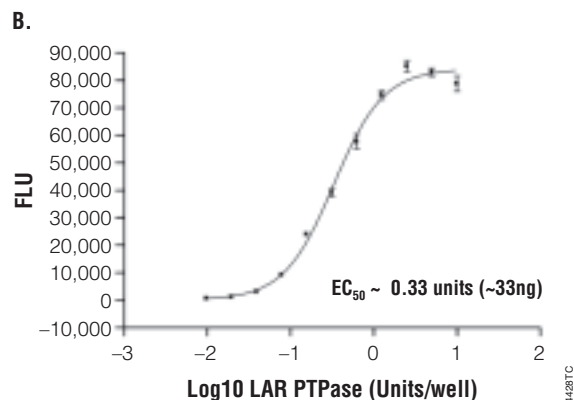
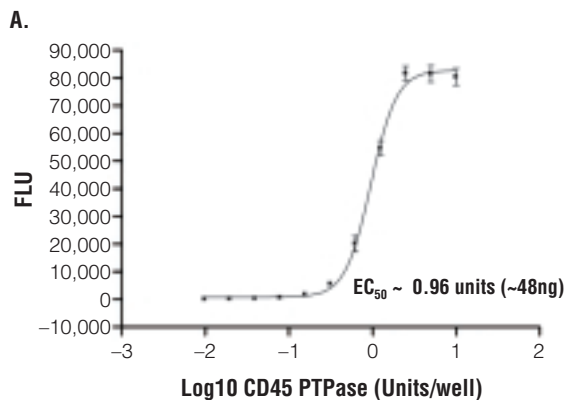


Figure 3. EC_{50} determinations for CD45 and LAR PTPases using the Tyrosine Phosphatase Assay.

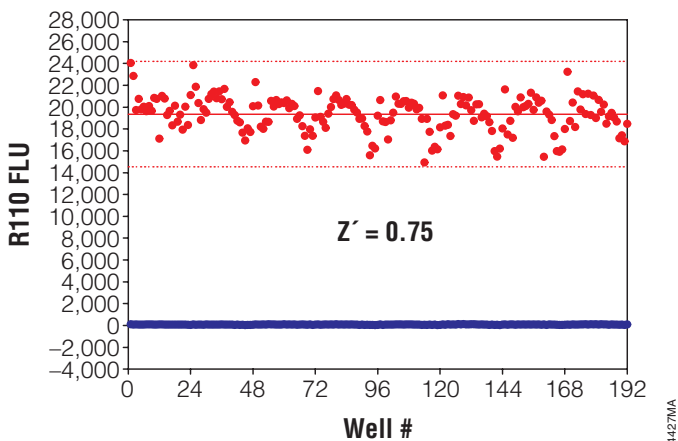


Figure 4. Z' -factor values of greater than 0.7 are routinely obtained using the ProFluor™ Tyrosine Phosphatase Assay. Results of a Z' -factor analysis are shown for a 384-well plate. The assay was performed manually according to Section III.B of the ProFluor™ Tyrosine Phosphatase Assay Technical Bulletin (#TB334) in a solid black, flat-bottom plate with 0.2 units LAR PTPase (red) and without enzyme (blue). Solid lines indicate the mean, and the dotted lines are ± 3 S.D.

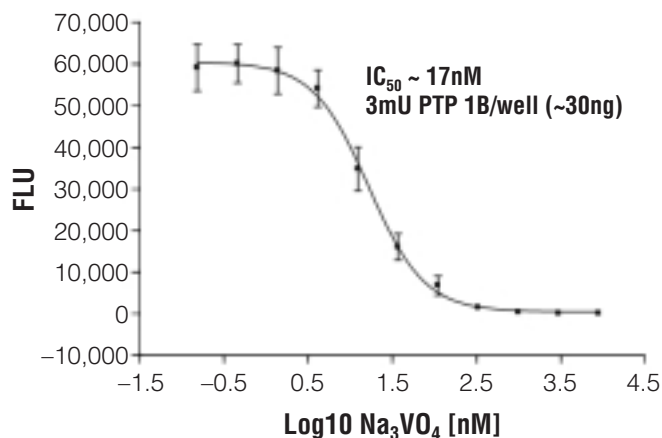


Figure 5. Accurate IC_{50} values. Results of a sodium vanadate titration in a solid black, flatbottom 96-well plate using 3mU PTP-1B/well (Calbiochem Cat. # 539735). Data points are the average of 8 determinations, and error bars are \pm S.D. Curve fitting was performed using GraphPad Prism® 4.0 sigmoidal dose response (variable slope software). IC_{50} values are similar to those reported in the literature (1,2).

Ordering Information

Product	Size	Cat. #
ProFluor™ Tyrosine Phosphatase Assay ^(a)	4 Plate	V1280
	8 Plate	V1281

Component listing for V1280:

- 12 μ l PTPase R110 Substrate, 10mM
- 12 μ l Control AMC Substrate, 10mM
- 240 μ l Protease Reagent
- 60 μ l Stabilizer Reagent
- 6ml 5X Reaction Buffer B
- 5ml 5X Termination Buffer C
- 200 μ l Sodium Vanadate

Component listing for V1281:

- 24 μ l PTPase R110 Substrate, 10mM
- 24 μ l Control AMC Substrate, 10mM
- 480 μ l Protease Reagent
- 120 μ l Stabilizer Reagent
- 12ml 5X Reaction Buffer B
- 10ml 5X Termination Buffer C
- 200 μ l Sodium Vanadate

References

1. Garton, A.J., Flint, A.J. and Tonks, N.K. (1996) Identification of p130cas as a substrate for the cytosolic protein tyrosine phosphatase PTP-PEST. *Mol Cell Biol*, **16**, 6408–18.
2. Schmidt, A. *et al.* (2002) Protein Tyrosine phosphatase activity regulates osteoclast formation and function: Inhibition by alendronate. *Proc. Natl. Acad. Sci. USA* **93**, 3068–73.

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^(a)Patent Pending.



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