Promega

Technical Bulletin

ProFluor® Tyrosine Phosphatase Assay

INSTRUCTIONS FOR USE OF PRODUCTS V1280 AND V1281.

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ProFluor® Tyrosine Phosphatase Assay

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1. Description

The ProFluor® Tyrosine Phosphatase Assay[a] measures the enzyme activity of tyrosine phosphatases using purified enzymes in a multiwell-plate format and involves "add, mix and read" steps only (Table 1). The assay begins with a standard phosphatase reaction performed in the provided reaction buffer, which contains a bisamide rhodamine 110 phosphopeptide substrate (PTPase R110 Substrate) and a Control AMC Substrate that serves as a control for compounds that may inhibit the protease. In this configuration, both the PTPase R110 Substrate and Control AMC Substrate are nonfluorescent (Figure 1, reference 1). Following the phosphatase reaction, addition of a protease solution simultaneously stops the phosphatase reaction and completely digests the nonphosphorylated PTPase R110 Substrate and the Control AMC substrate, producing highly fluorescent rhodamine 110 and AMC. The phosphorylated substrate, however, is resistant to digestion by the Protease Reagent and remains nonfluorescent. Thus, the measured fluorescence intensity in the assay correlates with phosphatase activity (Figure 3). The fluorescent signal is very stable (<20% change of fluorescence intensity over 4 hours), allowing batch-plate reading (Figure 2). The assay produces Z´-factor values greater than 0.7 in either 96-well

(data not shown) or 384-well plate formats (Figure 4), and it identifies known phosphatase inhibitors and can be used to identify inhibitors in a screen of library compounds. The assay produces IC_{50} values for known inhibitors that are comparable to those reported in literature (Figure 5, references 2–4).

Table 1. General Assay Format for 96-Well Plates and 384-Well Plates.

		Volume Per Well	
Step	**Description**	**96-Well**	**384-Well**
1.	Dilute tyrosine phosphatase in Reaction Buffer and add to well.	25µl	5µl
2.	Dilute PTPase R110 Substrate and Control AMC Substrate in Reaction Buffer and add to well.	25µl	5µl
3.	Mix plate and incubate for 60 minutes at room temperature (22–25°C).		
4.	Add Protease Solution.	25µl	5µl
5.	Mix plate and incubate for 30 minutes at room temperature.		
	Note: The protease reaction is temperature sensitive. We strongly recommend performing this reaction at 22–25°C. Increasing or decreasing the temperature will change the dynamic range of the assay.		
6.	Add Stabilizer Solution.	25µl	5µl
7.	Mix plate and read fluorescence at 485/530nm (R110) and 360/460nm (AMC).		

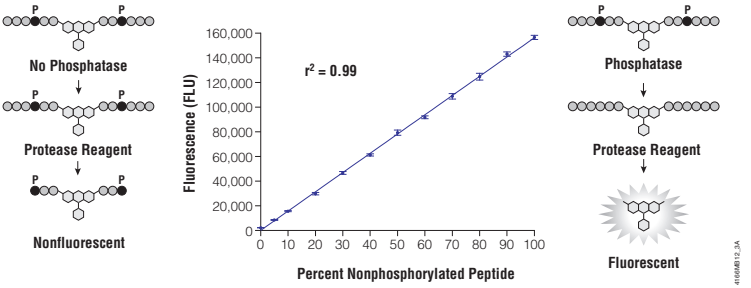

Figure 1. Effect of phosphopeptide content on fluorescence intensity. The graph shows the average FLU (n = 8) obtained after a 90-minute Protease Reagent digestion using mixtures of nonphosphorylated S/T PPase R110 Substrate and phosphorylated S/T PPase R110 Substrate as indicated (5). The total peptide concentration was 5µM in 50µl of Reaction Buffer B to which 25µl of Protease Reagent diluted in Termination Buffer B was added. (FLU = Fluoresence Light Unit; excitation = wavelength 485nm, emission = wavelength 530nm; $r^2 = 0.999$). Dark circles in the cartoon represent phosphorylated amino acids.

2. Product Components and Storage Conditions

Product	Size	Cat#
ProFluor® Tyrosine Phosphatase Assay	4 plate	V1280

Each system contains sufficient reagents for 4 × 96 assays in 96-well plates (100µl/assay) or 5 × 384 assays (20µl/assay) in 384-well plates. Includes:

- 12µl PTPase R110 Substrate
- 12µl Control AMC Substrate
- 6ml 5X Reaction Buffer B
- 5ml 5X Termination Buffer C
- 240µl Protease Reagent
- 60µl Stabilizer Reagent
- 200µl Sodium Vanadate, 100mM

2. Product Components and Storage Conditions (continued)

Product	Size	Cat.#
ProFluor® Tyrosine Phosphatase Assay	8 plate	V1281

Each system contains sufficient reagents for 8 x 96 assays in 96-well plates (100µl/assay) or 10 x 384 assays (20µl/assay) in 384-well plates. Includes:

- 24µl PTPase R110 Substrate
- 24µl Control AMC Substrate
- 12ml 5X Reaction Buffer B
- 10ml 5X Termination Buffer C
- 480µl Protease Reagent
- 120µl Stabilizer Reagent
- 200µl Sodium Vanadate, 100mM

Storage Conditions: The system should be stored at –20°C. The PTPase R110 Substrate and Control AMC Substrate should be protected from light. For best results, make solutions fresh and use immediately. System components should be thawed at room temperature, well mixed, and then returned to –20°C as soon as possible.

3. Protocols for ProFluor® Tyrosine Phosphatase Assay

Materials to Be Supplied by the User

- opaque-walled multiwell plates (e.g., MicroFluor® 2, black 96-well plates; Thermo Electron Cat.# 7805)
- multichannel pipette or automated pipetting station
- plate shaker (DYNEX MICRO-SHAKER®II or equivalent)
- fluorometer capable of reading multiwell plates, with filters for detecting both R110 and AMC fluorescence
- purified protein tyrosine phosphatase

Assay Overview

The assay begins with a phosphatase reaction using 5µM of PTPase R110 Substrate in 1X Reaction Buffer B [40mM Tris-HCl (pH 7.5), 0.1mg/ml BSA]. In this bisamide configuration, the PTPase R110 Substrate demonstrates very little fluorescence. Following dephosphorylation, a protease reaction is initiated in a buffer that also terminates the phosphatase reaction. After completion of the protease reaction, the plate can be read immediately or stabilized by adding Stabilizer Reagent, which terminates the protease reaction. Under conditions where there is no phosphatase activity, the peptide remains phosphorylated, which effectively blocks the activity of the protease. This results in very low fluorescence of the bisamide PTPase R110 Substrate. However, in the presence of active phosphatase, the phosphopeptide substrate will be dephosphorylated and the protease will remove all of the amino acids from the peptide substrate

and liberate the highly fluorescent rhodamine 110. Thus, with increasing amounts of phosphatase activity, a change from low fluorescence to high fluorescence is observed. The inclusion of the Control AMC Substrate helps to identify any false positives in the assay (protease inhibitors that mimic phosphatase inhibitors on fluorescence output). The control substrate can be included directly into the assay format, or hits can be rescreened against the protease with the control substrate.

We strongly recommend that users perform a phosphatase titration to determine the optimal amount of phosphatase to use for screening. This titration will also determine if the enzyme preparation contains any components that negatively affect the assay. We also recommend minimizing any reducing reagents in the assay. However, we have tested the protease in as much as 100µM of DTT or β-mercaptoethanol and have observed only minimal effects on protease activity. The assay has also been tested in reactions containing up to 2% DMSO (a common compound vehicle) with only minimal effects on performance. A sample phosphatase titration protocol is provided has been successfully used with PTP-1B (Calbiochem Cat.# 539735), CD45 PTPase (Calbiochem Cat.# 217614), YOP-51 (Calbiochem Cat.# 539734), LAR PTPase (Calbiochem Cat.# 539731), and T-cell PTPase (Calbiochem Cat.# 539732). Titration results are shown in Figure 3.

3.A. Tyrosine Phosphatase Titration (one 96- or 384-well plate)

Reagent Preparation

Prepare the following solutions before performing the assay.

Component		96-Well	384-Well
Phosphatase Dilution Solution			
	5X Reaction Buffer B	600µl	480µl
	NANOPure® Water to a volume of:	**3ml**	**2.4ml**
Peptide Solution			
	5X Reaction Buffer B	600µl	480µl
	PTPase R110 Substrate	3µl	2.4µl
	Control AMC Substrate	3µl	2.4µl
	NANOpure® Water to a volume of:	**3ml**	**2.4ml**
Protease Solution			
	5X Termination Buffer C	600µl	480µl
	Protease Reagent	60µl	48µl
	Sodium Vanadate, 100mM	9µl	7.2µl
	NANOpure® Water to a volume of:	**3ml**	**2.4ml**

3.A. Tyrosine Phosphatase Titration (one 96- or 384-well plate; continued)

Stabilizer Solution

5X Termination Buffer C	600µl	480µl
Stabilizer Reagent	15µl	12µl
Sodium Vanadate, 100mM	9µl	7.2µl
NANOpure® Water to a volume of:	**3ml**	**2.4ml**

96-Well Plate Protocol

1. Add 25µl of Phosphatase Dilution Solution to columns 1 through 11 of a 96-well plate. Solid black plates provide the best signal-to-noise ratios, although solid-white plates can also be used.
2. Dilute Phosphatase into 0.5ml Phosphatase Dilution Solution. Mix. Add 50µl to wells in column 12. Serially dilute with a multichannel pipettor by transferring 25µl to column 11, pipetting up and down to mix and transferring 25µl to column 10. Repeat for columns 9 through 2. Discard the final 25µl and do not dilute the phosphatase into column 1 (this column contains the no enzyme control wells). All wells should now have 25µl.
3. Add 25µl of Peptide Solution to all wells. This initiates the phosphatase reaction. All wells should now have 50µl.
4. Mix plate and incubate for 60 minutes at room temperature (22–25°C).
5. Add 25µl of Protease Solution to all wells. All wells should now have 75µl.
6. Mix plate and incubate for 30 minutes at room temperature.

   Note: The protease reaction is temperature sensitive. We strongly recommend performing this reaction at 22–25°C. Increasing or decreasing the temperature will change the dynamic range of the assay.
7. Add 25µl of Stabilizer Solution to all wells. All wells should now have 100µl.
8. Mix plate and read at excitation 485nm and emission 530nm (R110 signal). Read plate at excitation 360nm and emission 460nm (AMC signal).

384-Well Plate Protocol

1. Add 5µl of Phosphatase Dilution Solution to columns 1 through 11, rows A through H of a 384-well plate. Solid black plates provide the best signal-to-noise ratios, although solid white plates can also be used.
2. Dilute phosphatase into 0.5ml Phosphatase Dilution Solution. Mix. Add 10µl to wells in column 12. Serially dilute with a multichannel pipettor by transferring 5µl to column 11, pipetting up and down to mix and transferring 5µl to column 10. Repeat for columns 9 through 2. Discard the final 5µl and do not dilute the phosphatase into column 1 (this column is the no-enzyme control wells). All wells should now have 5µl.

3. Add 5µl of Peptide Solution to all wells. This initiates the phosphatase reaction. All wells should now have 10µl.
4. Mix plate and incubate for 60 minutes at room temperature (22–25°C).
5. Add 5µl of Protease Solution to all wells. All wells should now have 15µl.
6. Mix plate and incubate for 30 minutes at room temperature.

   Note: The protease reaction is temperature sensitive. We strongly recommend performing this reaction at 22–25°C. Increasing or decreasing the temperature will change the dynamic range of the assay.
7. Add 5µl of Stabilizer Solution to all wells. All wells should now have 20µl.
8. Mix plate and read at excitation 485nm and emission 530nm (R110 signal). Read plate at excitation 360snm and emission 460nm (AMC signal).

3.B. Determining Z´-Factor Values (one 96- or 384-well plate)

Choose an amount of enzyme from the phosphatase titration protocol that results in approximately 85–90% of the maximum signal in the assay (85–90% dephosphorylation). Using less phosphatase is possible; however, the dynamic range of the assay will narrow and %CV's will increase.

Reagent Preparation

Component	96-Well	384-Well
Phosphatase Solution		
5X Reaction Buffer B	400µl	320µl
purified tyrosine phosphatase (determined by titration)	Xµl	Xµl
NANOPure® Water to a volume of:	**2ml**	**1.6ml**
Control Buffer (no enzyme)		
5X Reaction Buffer B	400µl	320µl
NANOPure® to a volume of:	**2ml**	**1.6ml**
Peptide Solution		
5X Reaction Buffer B	600µl	480µl
PTPase R110 Substrate	3µl	2.4µl
Control AMC Substrate	3µl	2.4µl
NANOpure® Water to a volume of:	**3ml**	**2.4ml**
Protease Solution		
5X Termination Buffer C	600µl	480µl
Protease Reagent	60µl	48µl
Sodium Vanadate, 100mM	9µl	7.2µl
NANOpure® Water to a volume of:	**3ml**	**2.4ml**

3.B. Determining Z´-Factor Values (one 96- or 384-well plate; continued)

Stabilizer Solution

5X Termination Buffer C	600µl	480µl
Stabilizer Reagent	15µl	12µl
Sodium Vanadate, 100mM	9µl	7.2µl
NANOpure® Water to a volume of:	**3ml**	**2.4ml**

Step	**96-well**	**384-well**
1. Add Phosphatase Solution to rows A through D (96-well plate) or rows A through H (384-well plate).	25µl	5µl
2. Add Control Buffer to rows E through H (for 96-well plate) or rows I through P (for 384-well plate).	25µl	5µl
3. Add Peptide Solution to all wells.	25µl	5µl
4. Mix plate and incubate for 60 minutes at room temperature (22–25°C).		
5. Add Protease Solution to all wells.	25µl	5µl
6. Mix plate and incubate for 30 minutes at room temperature.		

Note: The protease reaction is temperature sensitive. We strongly recommend performing this reaction at 22–25°C. Increasing or decreasing the temperature will change the dynamic range of the assay.

7. Add Stabilizer Solution to all wells.	25µl	5µl
8. Mix plate and read at excitation 485nm and emission 530nm (R110 signal). Read plate at excitation 360nm and emission 460nm (AMC signal).		

3.C. Screening for Tyrosine Phosphatase Inhibitors (one 96- or 384-well plate)

Choose an amount of enzyme from the phosphatase titration protocol that results in approximately 85–90% of the maximum signal in the assay (85–90% dephosphorylation). Using less enzyme is possible; however, the dynamic range of the assay will narrow and %CVs will increase.

Reagent Preparation

Prepare the following solutions before performing the assay.

Component	96-Well	384-Well
Phosphatase Solution (1.25X)		
5X Reaction Buffer B	600µl	480µl
purified tyrosine phosphatase (determined by titration)	Xµl	Xµl
NANOPure® Water to a volume of:	**2.4ml**	**1.9ml**
Control Buffer (no enzyme)		
5X Reaction Buffer B	50µl	40µl
NANOPure® to a volume of:	**0.25ml**	**0.2ml**
Peptide Solution		
5X Reaction Buffer B	600µl	480µl
PTPase R110 Peptide Substrate	3µl	2.4µl
Control AMC Substrate	3µl	2.4µl
NANOpure® Water to a volume of:	**3ml**	**2.4ml**
Protease Solution		
5X Termination Buffer C	600µl	480µl
Protease Reagent	60µl	48µl
Sodium Vanadate, 100mM	9µl	7.2µl
NANOpure® Water to a volume of:	**3ml**	**2.4ml**
Stabilizer Solution		
5X Termination Buffer C	600µl	480µl
Stabilizer Reagent	15µl	12µl
Sodium Vanadate, 100mM	9µl	7.2µl
NANOpure® Water to a volume of:	**3ml**	**2.4ml**

3.C. Screening for Tyrosine Phosphatase Inhibitors (one 96- or 384-well plate; continued)

Step	96-well	384-well
1. Add test compound to each well, except for 16 control wells that should receive only the vehicle (vehicle-only control).	5µl	1µl
2. Add Phosphatase Solution to each well, except for 8 of the control wells that should receive Control Buffer (no enzyme).	20µl	4µl
3. Add Peptide Solution to all wells.	25µl	5µl
4. Mix plate and incubate for 60 minutes at room temperature (22–25°C).		
5. Add Protease Solution to all wells.	25µl	5µl

Note: The protease reaction is temperature sensitive. We strongly recommend performing this reaction at 22–25°C. Increasing or decreasing the temperature will change the dynamic range of the assay.

6. Mix plate and incubate for 30 minutes at room temperature.
7. Add Stabilizer Solution to all wells. 25µl 5µl
8. Mix plate and read at excitation 485nm and emission 530nm (R110 signal). Read plate at excitation 360nm and emission 460nm (AMC signal).

3.D. Determining IC_{50} Values (one 96- or 384-well plate)

Choose an amount of enzyme from the phosphatase titration protocol that results in approximately 85–90% of the maximum signal in the assay (85–90% dephosphorylation). Using less enzyme is possible; however, the dynamic range of the assay will narrow and %CVs will increase.

Reagent Preparation

1. Perform threefold serial dilution of 10X concentrated inhibitor in a separate 96-well round-bottom plate in 10% DMSO (or an equivalent vehicle) leaving one well as a vehicle-only control (no inhibitor).

   EXAMPLE: Add 66µl of 10% DMSO to columns 1–11 in one row of the plate. Add 99µl of the highest concentration of 10X inhibitor to be tested into column 12. Serially dilute with a pipettor by transferring 33µl to column 11, pipetting up and down to mix and transferring 33µl to column 10. Repeat for columns 9 through 2. Discard the final 33µl and do not dilute the inhibitor into column 1 (this column is the no-inhibitor control). All wells should now have 66µl.

2. **For 96-well plate:** Transfer 5µl of the appropriate concentration of 10X diluted inhibitor (from dilution series performed in step 1) to the appropriate wells of a solid-black 96-well plate (columns 1–12, rows A–H). Column 1 should be your vehicle-only control, with columns 2–12 being your dilution series of 10X inhibitor (column 12 being the highest concentration).

   Note: The final concentration of DMSO (or equivalent vehicle) should be 1% in a 50µl phosphatase reaction. The final concentration of 10X inhibitor in each well should be 1X in a 50µl phosphatase reaction.

   For 384-well plate: Transfer 1µl of the appropriate concentration of 10X diluted inhibitor (from dilution series performed in Step 1) to the appropriate wells of a solid-black 384-well plate (columns 1–12, rows A–H). Column 1 should be your vehicle-only control, with columns 2–12 being your dilution series of 10X inhibitor (column 12 being the highest concentration).

   Note: The final concentration of DMSO (or equivalent vehicle) should be 1% in a 10µl phosphatase reaction. The final concentration of 10X inhibitor in each well should be 1X in a 10µl phosphatase reaction.

3. Prepare the following solutions before performing the assay.

Component	96-Well	384-Well
Phosphatase Solution (1.25X)		
5X Reaction Buffer B	600µl	480µl
purified tyrosine phosphatase (determined by titration)	Xµl	Xµl
NANOPure® Water to a volume of:	**2.4ml**	**1.9ml**
Peptide Solution		
5X Reaction Buffer B	600µl	480µl
PTPase R110 Substrate	3µl	2.4µl
Control AMC Substrate	3µl	2.4µl
NANOpure® Water to a volume of:	**3ml**	**2.4ml**
Protease Solution		
5X Termination Buffer C	600µl	480µl
Protease Reagent	60µl	48µl
Sodium Vanadate, 100mM	9µl	7.2µl
NANOpure® Water to a volume of:	**3ml**	**2.4ml**
Stabilizer Solution		
5X Termination Buffer C	600µl	480µl
Stabilizer Reagent	15µl	12µl
Sodium Vanadate, 100mM	9µl	7.2µl
NANOpure® Water to a volume of:	**3ml**	**2.4ml**

3.D. Determining IC_{50} Values (one 96- or 384-well plate; continued)

Step	96-well	384-well
4. Inhibitor titration series (previously added; see Steps 1 and 2 above).	5µl	1µl
5. Add Phosphatase Solution to each well (columns 1–12, rows A–H).	20µl	4µl
6. Add Peptide Solution to each well (columns 1–12, rows A–H).	25µl	5µl
7. Mix plate and incubate for 60 minutes at room temperature (22–25°C).		
8. Add Protease Solution to all wells.	25µl	5µl
9. Mix plate and incubate for 30 minutes at room temperature.		

Note: The protease reaction is temperature sensitive. We strongly recommend performing this reaction at 22–25°C. Increasing or decreasing the temperature will change the dynamic range of the assay.

10. Add Stabilizer Solution to all wells.	25µl	5µl

11. Mix plate and read at excitation 485nm and emission 530nm (R110 signal). Read plate at excitation 355nm and emission 460nm (AMC signal).

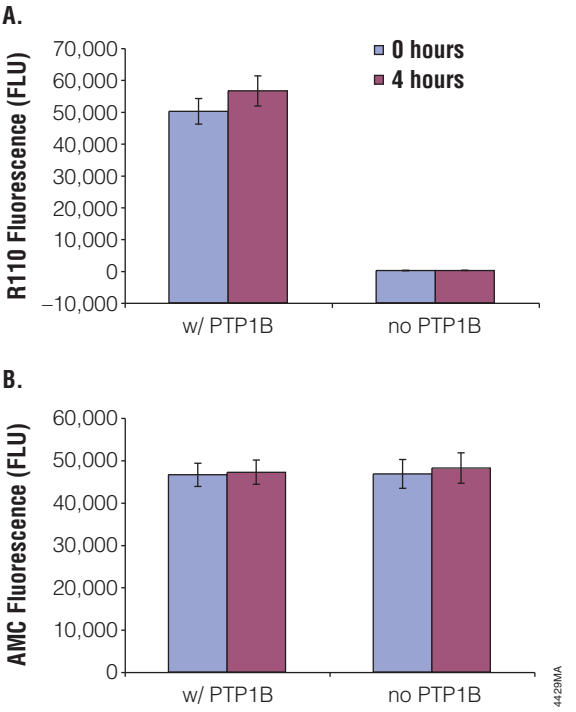

Figure 2. Stable fluorescent signal allows high-throughput batch processing. The bar chart shows averages ± S.D. of FLU values (n = 192) collected from an assay performed in a solid-black, flat-bottom 384-well plate using 1.2mU (~12ng) PTP-1B (Calbiochem Cat.# 539735) as described in Section 3.B, with and without enzyme, at time 0 and 4 hours later. The R110 and AMC signals increased less than 15% in four hours.

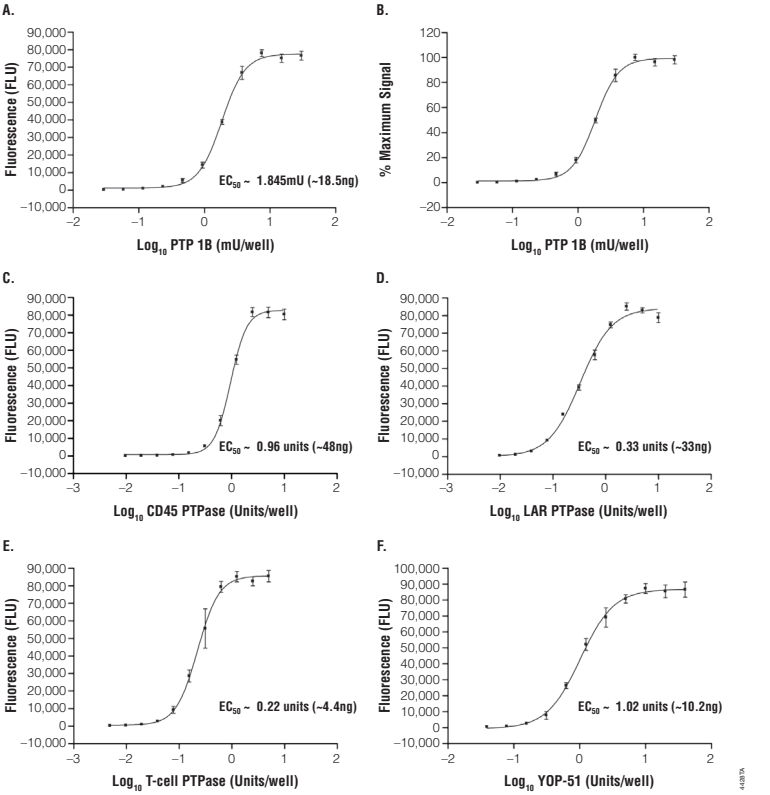

Figure 3. PTPase activity correlates with R110 fluorescence output. Results of titration curves that were performed according to the phosphatase titration protocol (Section 3.A) in solid-black, flat-bottom 96-well plates. **Panels A** and **B** show the results of a PTP-1B titration (Calbiochem Cat. # 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 ±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). Panels C, D, E**, and **F** show PTPase titrations with CD45 PTPase (Calbiochem Cat.# 217614), LAR PTPase (Calbiochem Cat.# 539731), T-cell PTPase (Calbiochem Cat.# 539732), and YOP-51 (Calbiochem Cat.# 539734). **Note:** Due to low baseline, the software program displays a negative Y-axis value.

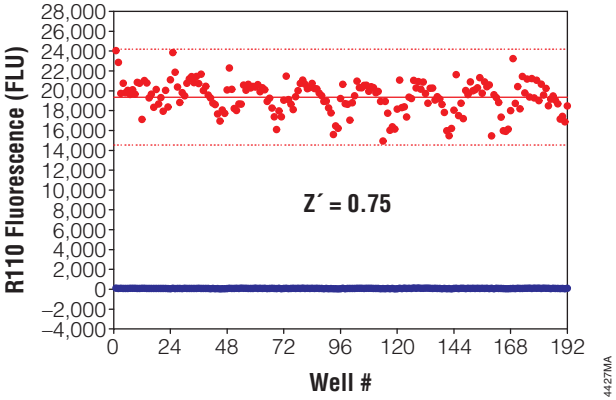

Figure 4. Z´-Factor value obtained in a 384-well plate. Results of a Z´-factor analysis are shown for a 384-well plate. The assay was performed manually according to Section 3.B in a solid black, flat-bottom plate with 0.2 units LAR PTPase (light gray) and without enzyme (black). Solid lines indicate the mean, and the dotted lines are ± 3 S.D. The Z´ factor for the assay under those conditions is 0.75 as calculated by the equation from Zhang *et al.* (1999) *J. Biomol. Screening* **4**, 67–73. **Note:** Due to low baseline, the software program displays a negative Y-axis value.

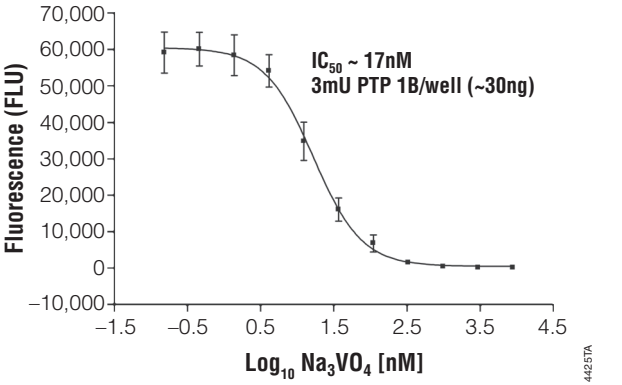

Figure 5. Accurate IC_{50} Values. Results of a sodium vanadate titration in a solid black, flat-bottom 96-well plate using 3mU PTP-1B/well (Calbiochem Cat. # 539735). The assay was performed as described in Section 3.D. Data points are the average of 8 determinations, and error bars are ± S.D. Curve fitting was performed using GraphPad Prism® 4.0 sigmoidal dose-response (variable slope). IC_{50} values are similar to those reported in the literature (2,3). **Note:** Due to low baseline, the software program displays a negative Y-axis value.

4. General Considerations

Temperature: Environmental factors that affect the rate of the protease and phosphatase reaction can influence fluorescence intensity. Temperature is one factor that affects the rate of the protease and thus the fluorescence in the assay. For consistent results, equilibrate assay plates and reagent to a constant temperature before performing the assay. Insufficient equilibration may result in a temperature gradient effect between the wells in the center and on the edge of the plates. The temperature gradient pattern may also depend on the position of the plate in the stack. Incubating the plate outside of the 22–25°C range may result in changes in the dynamic range of the assay.

Solvents and Additives: The chemical environment of the protease reaction will affect the enzymatic rate and thus fluorescence intensity. Some solvents used for the various chemical compounds tested may interfere with the protease reaction. Interference with the protease reaction can be determined by assaying a parallel set of control wells without phosphatase and measuring the AMC fluorescence. We have tested dimethylsulfoxide (DMSO), a commonly used vehicle to solubilize organic chemicals, at a final concentration of 2% in the assay and found that it had minimal effect on the assay. Standard phosphatase preparations are potentially another source of interfering chemicals. We have tested 100µM β-mercaptoethanol, 100µM DTT, and 1% glycerol in the assay, and none had a significant effect on fluorescence (Table 2).

Table 2. Effect of Solvents and Additives on Fluorescence.

Chemical	Concentration in Phosphatase Reaction	Reduction in Signal
DMSO	2%	<25%
DTT	100µM	<15%
β-mercaptoethanol	100µM	<3%
glycerol	1%	<3%

Plate Recommendations: We recommend using standard opaque-walled multiwell plates suitable for fluorescence measurements. Black plates provide a better signal-to-noise ratio, while white plates provide greater fluorescence. Round bottom plates also provide greater fluorescence than flat bottom plates.

Specific Results with Low False-Hit Rate and Protease Inhibition: Minimizing the number of false hits is important, and we have addressed this issue in the ProFluor® Phosphatase Assays. There are four possible outcomes from the combined effect of compounds on the phosphatase and protease when using the ProFluor® Tyrosine Phosphatase Assay (Table 3).

A compound that only inhibits the phosphatase will decrease the R110 fluorescent signal (530nm), as will a compound that only inhibits the protease.

To prevent protease inhibitors from being picked as "hits" during a screen, the AMC fluorescent signal (460nm) should be examined carefully where a decrease in R110 fluorescence is observed. If the AMC fluorescent signal is as strong as control wells that have active phosphatase, then the compound is not inhibiting the protease. However if the AMC fluorescence is decreased, then the compound is inhibiting the protease, and no call can be made with respect to the compound's effect on the phosphatase. Titrating screening hits that decreased R110 fluorescence will allow determination of accurate IC_{50} values.

Table 3. Possible Outcomes of the ProFluor® Tyrosine Phosphatase Assay.

Test Compound Inhibits:	AMC Fluorescence 460nm	R110 Fluorescence 527nm
Phosphatase Only	↑	↓
Protease Only	↓	↓
Phosphatase and Protease	↓	↓
Neither Enzyme	↑	↑

Up arrows (↑) indicate high fluorescent values; down arrows (↓) indicate low fluorescent values.

5. References

1. Leytus, S.P, Mehado, L.L. and Mangel, W.F (1983) Rhodamine-based compounds as fluorogenic substrates for serine proteases. *Biochem. J.* **209**, 299–307.
2. Garton, A.J., Flint, A.J. and Tonks, N.K. (1996) Identification of $p130^{cas}$ as a substrate for the cytosolic protein tyrosine phosphatase PTP-PEST. *Mol. Cell. Biol.* **16**, 6408–18.
3. Schmidt, A. *et al.* (1996) Protein tyrosine phosphatase activity regulates osteoclast formation and function: Inhibition by alendronate. *Proc. Natl. Acad. Sci. USA* **93**, 3068–73.
4. Guo, X-L. *et al.* (2002) Probing the molecular basis for potent and selective protein tyrosine phosphatase 1B inhibition. *J. Biol. Chem.* **277**, 41014–22.
5. *ProFluor® Ser/Thr PPase Assay Technical Bulletin* #TB324, Promega Corporation.

6. Composition of Buffers and Solutions

5X Reaction Buffer B

200mM Tris-HCl (pH 7.5)
0.5mg/ml BSA

5X Termination Buffer C

200mM Tris-HCl (pH 7.5)
0.5mg/ml BSA

7. Related Products

Protein Kinase Assay Systems

Product	Size	Cat.#
Kinase-Glo® Luminescent Kinase Assay	10ml	V6711
	10 × 10ml	V6712
	100ml	V6713
	10 × 100ml	V6714
Kinase-Glo® Plus Luminescent Kinase Assay	10ml	V3771
	10 × 10ml	V3772
	100ml	V3773
	10 × 100ml	V3774
ProFluor® PKA Assay	4 plate	V1240
	8 plate	V1241
ProFluor® Src-Family Kinase Assay	4 plate	V1270
	8 plate	V1271

PepTag® Non-Radioactive Protein Kinase Assays

Product	Size	Cat.#
PepTag® Non-Radioactive PKC Assay	120 reactions	V5330
PepTag® Non-Radioactive cAMP-Dependent Protein Kinase Assay	120 reactions	V5340

SignaTECT® Protein Kinase Assay Systems

Product	Size	Cat.#
SignaTECT® cAMP-Dependent Protein Kinase (PKA) Assay System	96 reactions	V7480
SignaTECT® Protein Kinase C (PKC) Assay System	96 reactions	V7470
SignaTECT® Protein Tyrosine Kinase (PTK) Assay System	96 reactions	V6480
SignaTECT® Calcium/Calmodulin-Dependent Protein Kinase (CaM KII) Assay System	96 reactions	V8161
SignaTECT® DNA-Dependent Protein Kinase Assay System	96 reactions	V7870
SignaTECT® cdc2 Protein Kinase Assay System	96 reactions	V6430

Non-Radioactive Phosphatase Assay Systems

Product	Size	Cat. #
ProFluor® Ser/Thr PPase Assay	4 plate	V1260
	8 plate	V1261
Serine/Threonine Phosphatase Assay System	96 reactions	V2460
Tyrosine Phosphatase Assay System	96 reactions	V2471

Protein Phosphatases

Product	Size	Cat.#
PPase-2A	25 units	V6311
PPase-2B	10 units	V6361

[a]Patent Pending.

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All prices and specifications are subject to change without prior notice.

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