Promega

Technical Bulletin

Tyrosine Phosphatase Assay System

INSTRUCTIONS FOR USE OF PRODUCT V2471.

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Tyrosine Phosphatase Assay System

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1. Description

The non-radioactive Tyrosine Phosphatase Assay System provides a fast, convenient and flexible alternative for measurement of protein tyrosine phosphatase activity. Using this system, the amount of free phosphate generated in a reaction is determined by measuring the absorbance of a molybdate:malachite green:phosphate complex (1–3). The Tyrosine Phosphatase Assay System allows use of a variety of buffer conditions and substrates, including naturally phosphorylated proteins or synthetic phosphopeptides.

Protein phosphorylation plays an important role in the regulation of many diverse cellular processes including differentiation, cell division, metabolism, contractility, fertilization and memory (for reviews, see references 4–19). These responses are regulated by a delicate balance between protein kinases and protein phosphatases. While protein kinases have been relatively easy to study by measuring the incorporation of radioactive phosphate into proteins or specific peptide substrates, characterization of protein phosphatases has proved

to be more difficult. One approach has involved the use of radiolabeled substrates for the phosphatases. However, this approach has several disadvantages: prelabeling of protein substrates with radioactive phosphate is time-consuming, the labeled substrate must be made repeatedly, and the resulting substrate rarely can be used at optimal concentrations.

Protein phosphatases can be divided into two main classes: 1) those that remove phosphate from proteins or peptides containing phosphotyrosine and 2) those that remove phosphate from proteins or peptides containing phosphoserine or phosphothreonine. To distinguish these two phosphatase classes, we have designed two assay systems. The Tyrosine Phosphatase Assay System contains two chemically synthesized phosphopeptides, END(pY)INASL (20) and DADE(pY)LIPQQG (21), which serve as substrates for many protein tyrosine phosphatases. The Serine/Threonine Phosphatase Assay System (Cat.# V2460) contains the chemically synthesized phosphopeptide, RRA(pT)VA, a peptide substrate that is compatible with several serine/threonine phosphatases such as the Protein Phosphatases 2A, 2B and 2C (22).

The effective range for detection of phosphate released during an assay using the Tyrosine Phosphatase Assay System is 200–4,000pmol of phosphate. While the lower end of this range is less sensitive than radioactive assays, the concentration of substrates and the working range of the assay are compatible with the V_{max} and K_m values of commonly used phosphatases using phosphopeptide substrates. This feature provides more accurate assessment of kinetic data, a benefit that cannot be claimed by the so-called more sensitive methods. Working in the higher range also reduces the possibility of detecting aberrant phosphatase activity due to promiscuity of certain phosphatases. At this range of sensitivity, low nanogram levels of phosphatases can be detected.

In addition to measuring phosphatase activity in partially fractionated and purified samples, the Tyrosine Phosphatase Assay System can also measure phosphatase activity in crude cell or tissue extracts. For this application, the high concentration of phosphate in these preparations is eliminated before performing the assay using the supplied Spin Columns, which rapidly and effectively remove free phosphate and other low molecular weight inhibitors from the sample. In addition, a unique Molybdate Dye Additive is combined with the Molybdate Dye Solution to aid in the solubilization of proteins exposed to the acid conditions of the Molybdate Dye Solution. Figure 1 provides an overview of the steps required to measure tyrosine phosphatase activity from partially purified enzyme preparations or cell/tissue extracts using this system.

2. Product Components and Storage Conditions

Product	Size	Cat.#
Tyrosine Phosphatase Assay System	96 reactions	V2471

Includes:

- 20ml Molybdate Dye Solution
- 200µl Molybdate Dye Additive
- 1ml Phosphate Standard, 1mM
- 1mg Tyr Phosphopeptide-1, END(pY)INASL
- 1mg Tyr Phosphopeptide-2, DADE(pY)LIPQQG
- 1 96-Well Plate (1/2 area, flat bottom)
- 4 Spin Columns, Reservoirs and Adaptors
- 40ml Sephadex® G-25
- 25ml Phosphate-Free Water

Storage Conditions: Store the Tyrosine Phosphatase Assay System at 4°C. The 96-Well Plate and Spin Columns may be stored at room temperature. Suspended phosphopeptide (Section 3.B) may be stored at 4°C or at –20°C.

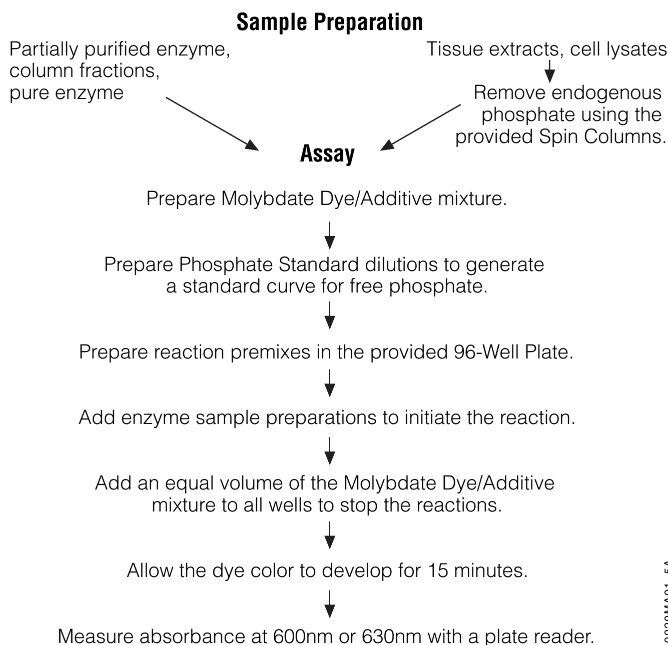


Figure 1. Overview of the Tyrosine Phosphatase Assay System protocol. The system can be used to measure phosphatase activity from partially purified enzyme preparations and tissue extracts/ cell lysates.

3. Before You Begin

3.A. Selection of Assay Format

Phosphatase assays may be performed in the supplied 96-Well Plate (Costar® Cat.# 3690) or in standard 96-well plates with good optical qualities. The 96-Well Plate provided with the system has a reduced well diameter and volume (190µl) that readily accommodates the standard 50µl reaction plus 50µl of the Dye/Additive mixture. Because of the well shape (tall and narrow), the supplied 1/2 area 96-Well Plate allows higher absorbance readings than standard 96-well plates. The convenient 96-well format of the supplied plate allows sample analysis under a variety of conditions using several time points.

Do not discard the supplied plate until all wells have been used. **Do not** reuse stained wells.

Standard plates typically have about half the optical density as the supplied 96-Well Plate at any given phosphate concentration and thus have a proportionally lower background for negative control samples. Because standard plates are not as sensitive, higher phosphate concentrations can be measured accurately (up to 4nmol of phosphate can be assayed before the optics of plate readers generate nonlinear responses). A comparison of the two plate types is shown in Figure 2. If you choose to use standard 96-well plates, perform reactions in 50–100µl volumes and add an equal volume of the Dye/Additive mixture as described in Section 4.A.

If a plate reader is unavailable, you may use larger volumes (e.g., 400µl of reaction mixture and 400µl of Dye/Additive mixture) so that cuvettes and a spectrophotometer can be used. Determine the minimum volume that can be used in a disposable cuvette (the dye will stain cuvettes). Half of this volume must be the Dye/Additive mixture (prepared in Section 4.A), and the other half can be reaction mix or a combination of reaction mix and water. The system contains enough Molybdate Dye Solution for about 50 reactions with a final volume of 800µl (reaction mixture plus Dye/Additive mixture).

3.B. Substrates

Phosphopeptides

Reconstitute the supplied Phosphopeptide with the supplied Phosphate-Free Water as described in Table 1. For general use, 5µl of this reconstituted substrate in a 50µl reaction is sufficient and will yield 100µM peptide, resulting in the release of up to 5nmol phosphate during the reaction. For kinetic data, perform a substrate concentration analysis by titrating the amount of substrate in the reaction over a range of 10–100µM, depending on the K_m value of the enzyme under study.

Note: Tyr Phosphopeptide-2 is very hygroscopic and may acquire some moisture during shipment. The peptide may be dried using a Speed-Vac® vacuum centrifuge without heat prior to reconstitution to obtain a more accurate concentration.

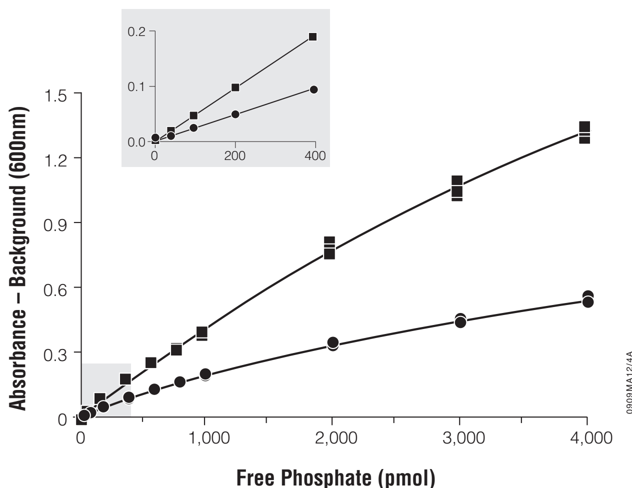


Figure 2. Comparison of absorbance results for Phosphate Standard when using the supplied 96-Well Plate (1/2 area, flat bottom; ■) and standard plates (●). Samples were measured in triplicate. The inset depicts the lower, linear detection range from the same experiments.

Table 1. Physical Characteristics of the Supplied Tyr Phosphopeptides.

Phosphopeptide	Sequence	Molecular Weight (Daltons)	Molecular Volume of Water Required for a 1mM Solution
Tyr Phosphopeptide-1	END(pY)INASL	1118	895µl
Tyr Phosphopeptide-2	DADE(pY)LIPQQG	1328	753µl

Phosphoproteins

Other substrates such as histone, casein or a natural substrate of the phosphatase of interest may also be used in this assay. Some of these proteins can be obtained commercially in a partially dephosphorylated state, but they usually require extensive dialysis to remove free phosphate before use. In addition, large proteins, proteins with multiple phosphorylation sites or proteins with limited solubility in strong acid will require some preliminary testing as described in Section 3.D.

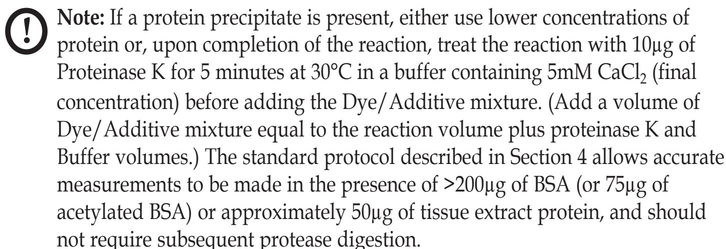
3.C. Buffers

As a family of enzymes, phosphatases have diverse optimal conditions; therefore, a universal buffer system cannot be provided. The pH optima of phosphatases are also substrate-dependent. For example, phosphatases appear to have higher V_{max} values with phosphopeptide substrates at lower pH values than with phosphoproteins. In addition, lower pH may result in higher K_m values. It is therefore recommended that a pH profile between 5.0 and 7.5 be performed when using the supplied phosphopeptides (see Figure 3, Panel B).

3.D. Testing of Reaction Components

Because the assay measures free phosphate, **phosphate buffers are not compatible with this system**. Reaction components that contain phosphate (e.g., glycerol phosphate) may interfere with analysis, depending on their concentration, purity and stability in strong acid. High concentrations of reductants may bleach the dye color over time, resulting in lower sensitivity. A final concentration of 0.02% β-mercaptoethanol has no effect on sensitivity; 0.05% β-mercaptoethanol has only a slight effect, and 0.1% β-mercaptoethanol results in an approximately 20% reduction in sensitivity. Many detergents at or below 0.1% can be used, but higher concentrations may generate high backgrounds. If high concentrations of detergent are required in the reaction, the background can be determined by including the corresponding concentration of the detergent in the Phosphate Standards (see Note following Step 1 in Section 4.B). In addition, some proteins such as casein are not soluble in strong acid and will precipitate when the Dye/Additive mixture is added, requiring an additional step (see Note at the end of this section).

To test the suitability of the various components, add individual or combined components at the highest concentration to be used in a total of 50µl. Add 50µl of Dye/Additive mixture (prepared in Section 4.A) and incubate at room temperature for 15 minutes (30 minutes if more than 5µg of protein is present). Prepare a control reaction containing 50µl of the supplied Phosphate-Free Water and 50µl of the Dye/Additive mixture. Components that remain yellow and do not cause precipitation are compatible with this system. The optical density at 600–630nm can be obtained to determine exact backgrounds. Backgrounds (plate plus water/Dye Solution) range between 0.06 and 0.13, depending on the plate type and wavelength used.



Note: If a protein precipitate is present, either use lower concentrations of protein or, upon completion of the reaction, treat the reaction with 10µg of Proteinase K for 5 minutes at 30°C in a buffer containing 5mM $CaCl_2$ (final concentration) before adding the Dye/Additive mixture. (Add a volume of Dye/Additive mixture equal to the reaction volume plus proteinase K and Buffer volumes.) The standard protocol described in Section 4 allows accurate measurements to be made in the presence of >200µg of BSA (or 75µg of acetylated BSA) or approximately 50µg of tissue extract protein, and should not require subsequent protease digestion.

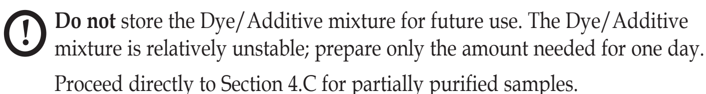
4. Protocols

The following protocol can be used to quantitate protein tyrosine phosphatase activity in cell lysates, tissue extracts, partially fractionated samples or purified enzymes. For partially purified preparations, no special sample preparation procedures are necessary as long as the free phosphate concentration is below 5µM (assuming 5µl sample size for a 50µl reaction).

This phosphatase assay accommodates a wide variety of buffers, reducing agents, detergents and glycerol. To ensure compatibility, however, pretest all reagents as described in Section 3.D.

4.A. Preparation of Dye

Determine the total reaction volume that you will use based on the particular assay format and sample number. Reactions prepared in the supplied 96-Well Plate or other standard plates require 50µl of the Dye/Additive mixture for each 50µl reaction. (Larger reaction volumes may be used.) Reactions to be analyzed in a spectrophotometer require approximately 400µl of the Dye/Additive mixture for each 400µl reaction. On the day of the experiment, prepare the Dye/Additive mixture (10µl of Molybdate Dye Additive per 1ml of Molybdate Dye Solution).



Do not store the Dye/Additive mixture for future use. The Dye/Additive mixture is relatively unstable; prepare only the amount needed for one day.

Proceed directly to Section 4.C for partially purified samples.

4.B. Preparation of Tissue Extracts or Cell Lysates

Cell lysates and tissue extracts contain millimolar concentrations of free phosphate that will interfere with the phosphatase assay. In addition, ATP at high concentrations can increase the background and lead to phosphorylation by contaminating kinases. It is therefore necessary to eliminate these components from samples.

The method of extraction may influence both the recovery of phosphatases and the presence of inhibitors of these enzymes.

Sample and Column Preparation

Materials to Be Supplied by the User
(Solution compositions are provided in Section 5.)

- 50ml disposable conical centrifuge tubes
- appropriate storage buffer (see Note following Step 1)
- Sephadex® G-25 storage buffer (for storing column)

1. Homogenize the tissue at 0–4°C for 30 seconds using 1g of tissue in 3ml of storage buffer.

   Note: The choice of storage buffer depends on several factors, including whether membrane-associated or cytoplasmic phosphatases are being examined. The storage buffer will generally include a reducing agent, a

4.B. Preparation of Tissue Extracts or Cell Lysates (continued)

chelator of divalent cations and various protease inhibitors. Up to 1% detergent (e.g., Triton® X-100) can be used to prepare membrane-associated phosphatases if the sample is diluted appropriately in the reaction mix. References 23–26 provide recipes for a variety of phosphatase storage buffers.

2. Centrifuge the homogenized lysate at 100,000 x *g* at 4°C for 1 hour to remove particulate matter.
3. Add 10ml of deionized water to a Spin Column and allow it to drain. To begin draining the column the first time, apply slight pressure to the Spin Column with a 10ml syringe or shake it briskly. The supplied Adaptor allows centrifugation in most standard 50ml disposable tubes. (A lid is not necessary.) Allow the Spin Column to drain into a waste container.
4. Resuspend the Sephadex® G-25 resin by rocking gently or pipetting with a wide-mouth pipette. **Do not** vortex or stir the resin with a magnetic stir bar.
5. Pipet 10ml of the resuspended Sephadex® slurry into the Spin Column and allow it to drain by gravity into a spare 50ml tube. Remove the flowthrough liquid from the tube.
6. Add 10ml of cold phosphatase storage buffer to the column.
7. Allow the column to drain by gravity, remove the flowthrough liquid from the tube, and then centrifuge at 600 x *g* for 5 minutes at 4°C using a spare 50ml tube to remove the remaining buffer surrounding the Sephadex® beads.
8. Place the Spin Column with Adaptor in the supplied Reservoir (50ml tube) and add 250µl of tissue extract or cell lysate. (A larger volume of sample may be used; see Note following Step 9.)
9. Centrifuge at 600 x *g* for 5 minutes at 4°C. The sample lysate in storage buffer will be in the bottom of the Reservoir in the original volume.

   Note: The sample flowthrough in the Reservoir should contain 4–10% of the endogenous phosphate. This reduced phosphate level should be low enough to perform most experiments with minimal background. If additional phosphate must be removed, pass the collected sample flowthrough through a second Spin Column. For determination of specific activities, measure the protein concentration of the phosphate-reduced sample(s).

   Using a larger sample volume in Step 8 will reduce the efficiency with which free phosphate is removed. For example, if 500µl of sample is used in Step 8, approximately 85–90% of the endogenous phosphate will be removed.

   Note: Spin Columns can be washed with at least 25ml of deionized water or phosphate-free buffer and used again following equilibration with Sephadex® G-25 storage buffer. Store used Spin Columns wet at 4°C.

4.C. Phosphatase Assay Protocol

1. Make appropriate phosphate standards by diluting the 1mM Phosphate Standard with the supplied Phosphate-Free Water. Dilute the Standard 1:20 to generate a solution containing 50pmol phosphate per microliter (50µM). Prepare wells containing 0, 100, 200, 500, 1,000 and 2,000pmol free phosphate and 1X reaction buffer in 50µl for use as a standard curve.

2. Prepare appropriate reaction premix solutions directly in the 96-Well Plate, excluding the enzyme sample. Do not create bubbles.

   Note: Reaction components may include substrate, phosphatase activators or inhibitors and a suitable buffer. The final reaction volume depends on the assay format. We recommend 50µl reactions for use with the supplied 1/2 area, flat-bottom 96-Well Plate; 50–100µl reactions for use with standard 96-well plates; and 400µl if using standard disposable cuvettes and a spectrophotometer in Step 7 of this section.

3. Place the 96-Well Plate at the desired temperature for 3 minutes.

   Note: One approach to controlling the reaction temperature is to place a glass dish containing a small amount of water in a water bath and allow the temperature to equilibrate. The 96-Well Plate can be placed in the equilibrated dish, where manipulations can be performed easily.

4. Start the reaction by adding the enzyme sample (1–35µl) to the wells containing the appropriate reaction solution and incubating the reactions for the desired time. Proper controls include reactions without enzyme sample and reactions without substrate, as well as complete reactions (substrate plus enzyme sample) that are terminated at time zero as described in Step 5.

5. Stop the reaction by adding 50µl of Molybdate Dye/Additive mixture prepared in Section 4.A. This mixture is a strong acid and will stop the enzymatic reaction so that accurate time points can be taken. Other termination solutions such as SDS should be used with caution as these reagents may react with the Molybdate Dye/Additive mixture and generate high backgrounds or lower the sensitivity of the assay.

   Note: Add 50µl of the Molybdate Dye/Additive mixture to wells containing the Phosphate Standard dilutions. If you are using standard 96-well plates, add an equal volume of the Dye/Additive mixture as described in Section 4.A.

6. Place the 96-Well Plate at room temperature and incubate for 15 minutes. (Incubate the 96-Well Plate for 30 minutes if more than 5µg of protein is present per reaction because high concentrations of protein delay color development.) Once color development is complete, the color remains stable for at least 2 hours. Thus, the addition of dye at various times during a timecourse experiment is acceptable as long as the appropriate development time at room temperature is allowed for the last time point.

4.C. Phosphatase Assay Protocol (continued)

7. Read the optical density of the samples using a plate reader with a 630nm or 600nm filter. The 630nm filter will give slightly higher values and higher backgrounds for both experimental and control samples.

Bubbles in the wells or water drops on the bottom of the plate will adversely affect the absorbance readings.

Table 2. General Approaches to the Initial Characterization of Phosphatases in Cell/Tissue Extracts and Column Fractions.

Enzyme	Phosphopeptide Substrate		Enzyme Activity in the Presence of Various Protein Phosphatase Inhibitors					
Protein Phosphatase Class	Phospho-tyrosine-containing Peptide	Phospho-threonine-containing Peptide	Vanadate	NaF	EDTA (no Mg^{2+})	EGTA (no Ca^{2+})	Okadaic Acid (50nm)	Trifluor-perazin
PTPases	++++	–	–	++++	++++	++++	++++	++++
PPTase-2A	–	++++	++++	–	++++	++++	–	++++
PPTase-2B	++	+++	++	–	++++	–	++++	–
PPTase-2C	–	++++	++++	–	–	++++	++++	++++

KEY: ++++, high activity; +++, moderately high activity; ++, moderate activity; +, low activity; –, very low to no detectable activity

5. Composition of Buffers and Solutions

Sephadex® G-25 storage buffer

10mM Tris (pH 7.5)
1mM EDTA
0.02% sodium azide

Phosphate Standard

1mM KH_2PO_4

6. References

1. Van Veldhoven, P.P. and Mannaerts, G.P. (1987) Inorganic and organic phosphate measurements in the nanomolar range. *Anal. Biochem.* **161**, 45–8.
2. Ekman, P. and Jager, O. (1993) Quantification of subnanomolar amounts of phosphate bound to seryl and threonyl residues in phosphoproteins using alkaline hydrolysis and malachite green. *Anal. Biochem.* **214**, 138–41.
3. Harder, K.W. *et al.* (1994) Characterization and kinetic analysis of the intracellular domain of human protein tyrosine phosphatase beta (HPTP beta) using synthetic phosphopeptides. *Biochem. J.* **298**, 395–405.
4. Cohen, P. (1989) The structure and regulation of protein phosphatases. *Annu. Rev. Biochem.* **58**, 453–508.
5. Hunter, T. *et al.* (1992) Receptor protein tyrosine kinases and phosphatases. *Cold Spring Harb. Symp. Quant. Biol.* **57**, 25–41.

6. Walton, K.M. and Dixon, J.E. (1993) Protein tyrosine phosphatases. *Annu. Rev. Biochem*. **62**, 101–20.

7. Brady-Kalnay, S.M. and Tonks, N.K. (1994) Protein tyrosine phosphatases: from structure to function. *Trends Cell Biol***. 4**, 73–6.

8. Shenolikar, S. (1994) Protein serine/threonine phosphatases-new avenues for cell regulation. *Annu. Rev. Cell Biol*. **10**, 55–86.

9. Hunter, T. (1995) Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell* **80**, 225–36.

10. Hooft van Huijsduijnen, R. (1998) Protein tyrosine phosphatases: counting the trees in the forest. *Gene* **225**, 1–8.

11. Goldberg, Y. (1999) Protein phosphatase 2A: who shall regulate the regulator? *Biochem. Pharmacol*. **57**, 321–8.

12. Denu, J.M. and Dixon, J.E. (1998) Protein tyrosine phosphatases: mechanisms of catalysis and regulation. *Curr. Opin. Chem. Biol*. **2**, 633–41.

13. Berndt, N. (1999) Protein dephosphorylation and the intracellular control of the cell number. *Front. Biosci*. **4**, D22–42.

14. Burke, T.R., Jr., and Zhang, Z.Y. (1998) Protein-tyrosine phosphatases: structure, mechanism, and inhibitor discovery. *Biopolymers* **47**, 225–41.

15. Oliver, C.J. and Shenolikar, S. (1998) Physiologic importance of protein phosphatase inhibitors. *Front. Biosci*. **3**, D961–72.

16. Stoker, A. and Dutta, R. (1998) Protein tyrosine phosphatases and neural development. *Bioessays* **20**, 463–72.

17. Keyse, S.M. (1998) Protein phosphatases and the regulation of MAP kinase activity. *Semin. Cell Dev. Biol*. **9**, 143–52.

18. Zhang, Z.Y. (1998) Protein-tyrosine phosphatases: biological function, structural characteristics, and mechanism of catalysis. *Crit. Rev. Biochem. Mol. Biol*. **33**, 1–52.

19. Hunter, T. (2000) Signaling—2000 and beyond. *Cell* **100**, 113–27.

20. Daum, G. *et al*. (1993) A general peptide substrate for protein tyrosine phosphatases. *Anal. Biochem*. **211**, 50–4.

21. Zhang, Z.Y. *et al*. (1993) Substrate specificity of the protein tyrosine phosphatases. *Proc. Natl. Acad. Sci. USA* **90**, 4446–90.

22. Donella Deana, A. *et al*. (1990) An investigation of the substrate specificity of protein phosphatase 2C using synthetic peptide substrates; comparison with protein phosphatase 2A. *Biochim. Biophys. Acta* **1051**, 199–202.

23. Tonks, N.K., Diltz, C.D. and Fischer, E.H. (1988) Purification of the major proteintyrosine-phosphatases of human placenta. *J. Biol. Chem*. **263**, 6722–30.

24. Cohen, P. *et al*. (1988) Protein phosphatase-1 and protein phosphatase-2A from rabbit skeletal muscle. *Methods Enzymol*. **159**, 390–408.

25. Zhao, Z. *et al*. (1993) Purification and characterization of a protein tyrosine hosphatase containing SH2 domains. *J. Biol. Chem*. **268**, 2816–20.

26. Zhao, Z. *et al*. (1994) Purification and characterization of PTP2C, a widely distributed protein tyrosine phosphatase containing two SH2 domains. *J. Biol. Chem*. **269**, 8780–5.

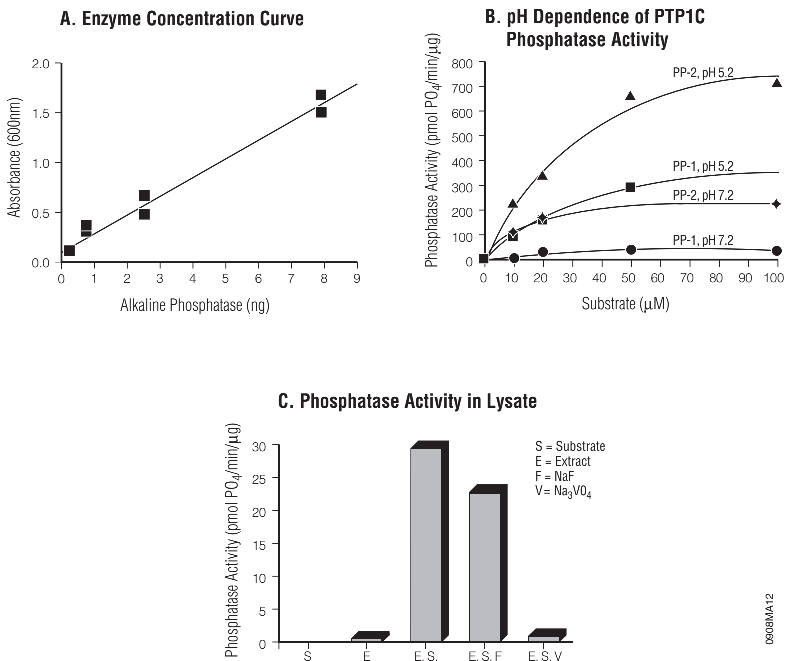


Figure 3. Typical data generated with the provided phosphopeptides. Purified enzymes as indicated were reacted for 5 minutes at 30°C with: (**Panel A**) 100µM Tyr Phosphopeptide-1 in Tris buffer (pH 7.3) and (**Panel B**) various concentrations Tyr Phosphopeptide-1 and -2 in 60mM sodium acetate (pH 5.2) or 50mM imidazole-HCl (pH 7.2). **Panel C** shows data obtained from A431 cell lysate that was passed once through the supplied Sephadex® G-25 Spin Column to remove free phosphate. The samples in Panel C were incubated for 45 minutes with or without substrate (100µM Tyr Phosphopeptide-1), 50mM sodium fluoride (NaF) and 1mM sodium vanadate. Phosphatase activity was substrate-dependent and was relatively insensitive to high concentrations of the serine/threonine phosphatase inhibitor, sodium fluoride, but was inhibited by the protein tyrosine phosphatase inhibitor, sodium vanadate. Abbreviations: PTP1C = Protein Tyrosine Phosphatase-1C; PP-1 = Tyr Phosphopeptide-1; PP-2 = Tyr Phosphopeptide-2.

7. Related Products

Product	Size	Cat.#
Serine/Threonine Phosphatase Assay System	96 reactions	V2460

Fluorescent Phosphatase Assay Systems

Product	Size	Cat.#
ProFluor® Ser/Thr PPase Assay	4-plate*	V1260
ProFluor® Tyrosine Phosphatase Assay	4-plate*	V1280

*Available in additional sizes.

Luminescent Universal Kinase Assays

Product	Size	Cat.#
Kinase-Glo® Max Luminescent Kinase Assay	10ml*	V6071
Kinase-Glo® Plus Luminescent Kinase Assay	10ml*	V3771
Kinase-Glo® Luminescent Kinase Assay	10ml*	V6711

*Available in additional sizes.

Fluorescent Kinase Assays

Product	Size	Cat.#
ProFluor® PKA Assay	4-plate*	V1240
ProFluor® Src-Family Kinase Assay	4-plate*	V1270

*Available in additional sizes.

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