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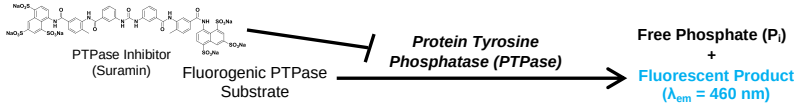
Protein Tyrosine Phosphatase Activity Assay Kit (Fluorometric)

05/17

(Catalog # K829-100; 100 Reactions; Store at -20°C)

I. Introduction:

Protein tyrosine phosphatases (PTPase(s), EC 3.1.3.48) are a diverse family of enzymes that catalyze the removal of a phosphate moiety from protein phosphotyrosine residues. Phosphorylation and dephosphorylation of tyrosine residues is a common post-translational modification that serves as a regulatory switch for many enzymes, receptors and signal transduction pathways. PTPs can be classified as intracellular (non-receptor) or receptor-like based upon their cellular localization, however all classical PTPs share a common catalytic mechanism utilizing a nucleophilic cysteine residue. As key regulators of cellular signaling cascades, PTPs affect cell growth, differentiation, migration and metabolism. Dysfunction of certain PTPs has been linked to several human diseases. For example, the intracellular tyrosine phosphatase PTP1B is a negative regulator of the insulin signaling cascade and has also been demonstrated to be dramatically overexpressed in human breast, colon and ovarian cancers. BioVision's PTPase Activity Assay Kit enables rapid measurement of PTPase activity, utilizing a fluorogenic protein phosphatase substrate that is converted into a highly fluorescent product (Ex/Em = 368/460 nm). A broad-spectrum PTPase inhibitor is provided for verification of specific activity in complex biological matrices, where serine/threonine phosphatases may contribute to substrate metabolism. Unlike ELISAs or malachite green-based approaches, the assay is homogeneous, continuous and does not require complicated sample processing or desalting to eliminate free phosphate. The assay is simple to perform, high-throughput adaptable and can detect a minimum of 0.1 mU PTPase activity.



II. Applications:

- Rapid assessment of PTPase activity in biological samples or recombinant PTPase preparations

III. Sample Type:

- Human or animal soft tissue (*i.e.* brain, liver, lung, etc.) homogenates
- Cultured cell lysates (adherent or suspension cells)
- Heterologously expressed recombinant protein tyrosine phosphatase preparations

IV. Kit Contents:

Components	K829-100	Cap Code	Part Number
PTPase Assay Buffer	25 ml	WM	K829-100-1
Disulfide Reducing Agent (DTT)	300 µl	Blue	K829-100-2
Fluorescence Standard	50 µl	Yellow	K829-100-3
PTPase Inhibitor (Suramin)	1 vial	Amber	K829-100-4
PTPase Substrate	1 vial	Red	K829-100-5
PTPase Positive Control	1 vial	Violet	K829-100-6

V. User Supplied Reagents and Equipment:

- Multiwell fluorescence microplate reader
- Precision multi-channel pipette and reagent reservoir
- 96-well plate with flat bottom (black or clear plates may be used)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the PTPase Assay Buffer to warm to room temperature prior to use. Read entire protocol before performing the assay procedure.

- **Disulfide Reducing Agent (DTT):** Provided as a 100 mM stock solution. Aliquot and store at -20°C, avoid repeated freeze/thaw cycles.
- **Fluorescence Standard:** Provided as a 5 mM stock solution in DMSO. Store at -20°C, stable for 5 freeze/thaw cycles.
- **PTPase Inhibitor (Suramin):** Reconstitute with 110 µl of dH_2O and vortex to yield a 10 mM stock solution. To prepare a 2 mM working solution (10X final concentration), mix 100 µl of the 10 mM stock solution and 400 µl of dH_2O. The 2 mM working solution should be stored at -20°C, protected from light and is stable for 3 freeze/thaw cycles.
- **PTPase Substrate:** Reconstitute with 44 µl of dH_2O to obtain a 500X stock solution. Aliquot and store at -20°C, protected from light. Avoid repeated freeze/thaw cycles.
- **PTPase Positive Control:** Reconstitute with 110 µl PTPase Assay Buffer. Aliquot and store at -80°C, avoid repeated freeze/thaw cycles. Once reconstituted, use PTPase Positive Control within 2 months.

VII. Protein Tyrosine Phosphatase (PTPase) Activity Assay Protocol:

1. **Sample Preparation:** Aliquot enough PTPase Assay Buffer for the number of reactions to be performed. Add Disulfide Reducing Agent (DTT) to PTPase Assay Buffer at a 1:50 ratio (20 µl of 100 mM DTT stock solution per 1 ml of PTPase Assay Buffer) immediately prior to use. Homogenize mammalian soft tissues (~50 mg) or pelleted, pre-washed cells (~5 x 10^6) in 500 µl ice-cold PTPase Assay Buffer (with DTT). Incubate the homogenate on ice for 5 min and centrifuge at 10,000 x *g* and 4°C for 15 min. Collect the supernatant and keep on ice until use (tissue homogenates and cell lysates can also be aliquoted and stored at -80°C for future experiments).

   Notes:
   - Always prepare fresh PTPase Assay Buffer with DTT. Once prepared, keep buffer with DTT on ice and use within 4 hrs.
   - The sample volume and/or dilution factor required can vary based upon the nature of the sample. For unknown samples, we suggest doing a pilot experiment by testing several amounts to ensure the readings are within the range of the standard curve.

- We recommend measuring sample protein concentration using the Bradford reagent (Cat. #K810) or a comparable protein assay.

2. **Standard Curve Preparation:** Dilute the Fluorescence Standard by adding 20 µl of the 5 mM stock to 980 µl PTPase Assay Buffer to obtain a 100 µM solution. Add 0, 2, 4, 6, 8, 12, 16 and 20 µl of the 100 µM solution into a series of wells and adjust the volume of each well to 100 µl with PTPase Assay Buffer, yielding 0, 200, 400, 600, 800, 1200, 1600 and 2000 pmol/well Fluorescence Standard.
3. **Reaction Mix:**
   a. Prepare assay reaction wells according to the table below. Add 2-20 µl of the test sample(s) to desired wells in a flat-bottom 96-well plate. In addition to test sample wells, prepare parallel inhibitor control wells (sample + 200 µM Suramin) using the Suramin 2 mM working solution. If desired, prepare a background control (no enzyme) well to correct for potential non-enzymatic substrate hydrolysis and positive control wells using the reconstituted PTPase Positive Control. Adjust the volume of all reaction wells to 80 µl/well with PTPase Assay Buffer (with DTT):

	Test Sample	+PTPase Inhibitor	Background Control	Positive Control
Sample	2–20 µl	2–20 µl	—	—
PTPase Positive Control	—	—	—	10 µl
Suramin 2 mM Solution (10X)	—	10 µl	—	—
PTPase Assay Buffer (With DTT)	to 80 µl	to 80 µl	80 µl	70 µl

   b. Preincubate the plate for 10 min at 25°C to allow the inhibitor to interact with sample PTPase. During the preincubation, prepare a 5X concentrated PTPase Substrate solution by diluting the reconstituted 500X PTPase Substrate stock at a 1:100 ratio. Prepare 20 µl of 5X PTPase Substrate solution for each reaction to be performed (for example, for 10 wells, mix 2 µl of 500X PTPase Substrate stock with 198 µl PTPase Assay Buffer).
   c. Start the reaction by adding 20 µl of the 5X PTPase Substrate solution to each reaction well using a multichannel pipette, yielding a final volume of 100 µl/well. *Do not add PTPase Substrate solution to the standard curve wells.*
4. **Measurement:** Immediately begin measuring the fluorescence at Ex/Em = 368/460 nm in kinetic mode for 30-45 min at 25°C. Ideal measurement time for the linear range may vary depending upon the sample (we recommend reading test sample fluorescence in kinetic mode). *The standard curve wells may be read in endpoint mode (Ex/Em = 368/460 nm).*
5. **Calculations:** For the Fluorescence Standard curve, subtract the 0 pmol/well reading from all standard readings, plot the background-subtracted values and calculate the slope. For sample reaction wells (including paired inhibitor control wells), choose two time points (t_1 and t_2) in the linear phase of the reaction progress curves, obtain the corresponding fluorescence values at those points (RFU_1 and RFU_2) and determine the change in fluorescence over the time interval: $\Delta F = RFU_2 - RFU_1$. Calculate the specific fluorescence generated by PTPase activity (denoted by C_S) by subtracting the suramin inhibitor control (ΔF_I) from the corresponding test sample (ΔF_S): $\boldsymbol{C_S = \Delta F_S - \Delta F_I}$. PTPase activity is obtained by applying the C_S values to the fluorescence standard curve to get B pmol of substrate metabolized during the reaction time.

$$\textbf{Protein Tyrosine Phosphatase (PTPase) Specific Activity} = \frac{\boldsymbol{B}}{\boldsymbol{\Delta T \times P}} = \textbf{pmol/min/mg} \equiv \textbf{µU/mg}$$

Where: $\mathbf{B}$ is the amount of metabolite produced, calculated from the standard curve (in pmol)
$\mathbf{\Delta T}$ is the linear phase reaction time $t_2 - t_1$ (in minutes)
$\mathbf{P}$ is the amount of protein added to the sample well (in mg)

PTPase Unit Definition: One unit of PTPase activity is the amount of enzyme that generates 1 µmole of fluorescent product per min by hydrolysis of 1 µmole PTPase Substrate at 25°C and pH 7.

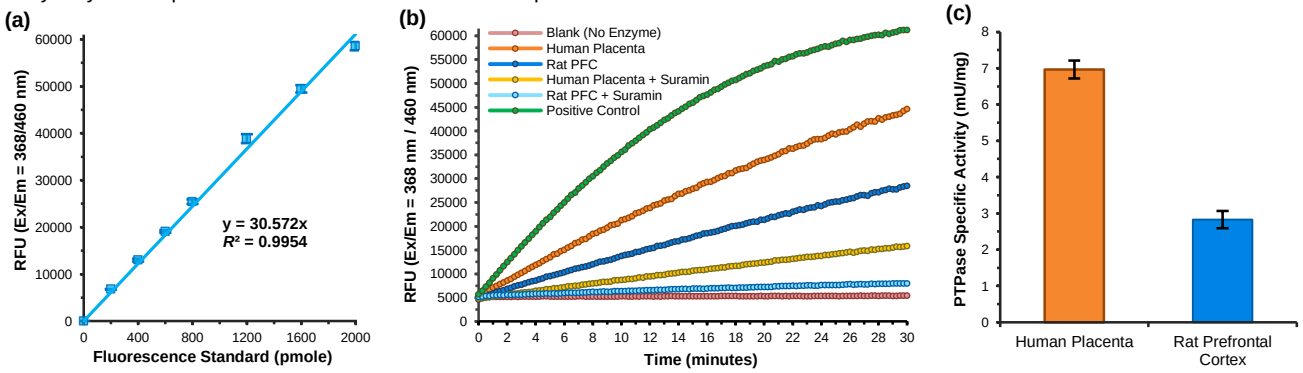


Figure: (a) Fluorescence Standard curve. One mole of Fluorescence Standard corresponds to the metabolism of one mole of PTPase Substrate with release of one mole free inorganic phosphate. **(b)** Reaction kinetics of PTPase Substrate metabolism in homogenates of human placenta (5 µg) and rat prefrontal cortex (10 µg) in the presence and absence of the PTPase inhibitor suramin. **(c)** Quantification of PTPase activity in samples (mean ± SEM of 4 or more independent replicates). Assays were performed according to the kit protocol.

VIII. RELATED PRODUCTS:

Acid Phosphatase Activity Assay Kit (K421)	Alkaline Phosphatase Activity Assay Kit (K422)	Human Recombinant PTP1B (6301)
Human Recombinant SHP-1 (6302)	Human Recombinant PTPN7 (7813)	Sodium Orthovanadate (9470)

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