ELISA PRODUCT INFORMATION & MANUAL

Protein Tyrosine Phosphatase Assay Kit *NBP2-59739*

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

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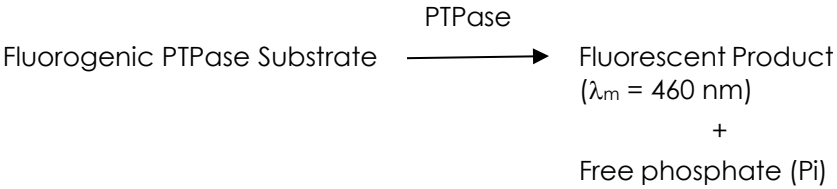
1. Overview

Protein Tyrosine Phosphatase Assay Kit (NBP2-59739) enables rapid measurement of protein tyrosine phosphatase (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.



Prepare Samples, Positive Control, Inhibitor Controls, Background Controls and Fluorescence Standards.

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Incubate plate for 10 minutes at 25°C to allow the inhibitor to interact with Sample PTPase.

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Start reaction by adding 20 µL of 5X PTPase Substrate to each well (apart from Fluorescence Standards).

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Measure fluorescence (Ex/Em = 368/460 nm) in kinetic mode for 30 - 45 minutes at 25°C (Standards may be read in end-point mode).

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
PTPase Assay Buffer	25 mL	-20°C	-20°C
Disulfide Reducing Agent (DTT)	300 µL	-20°C	-20°C
Fluorescence Standard	50 µL	-20°C	-20°C
PTPase Inhibitor (Suramin)	1 vial	-20°C	-20°C
PTPase Substrate	1 vial	-20°C	-20°C
PTPase Positive Control	1 vial	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em = 368/460 nm
- 96 well plate with flat bottom (black or clear plates may be used)
- Dounce homogenizer
- We recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as general use cocktail.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 PTPase Assay Buffer

Ready to use as supplied. Allow to warm to room temperature before use.

5.2 Disulfide Reducing Agent (DTT)

Ready to use as supplied. Provided as a 100 mM stock solution. Aliquot and store at -20°C, avoid repeated freeze/thaw cycles.

5.3 Fluorescence Standard

Ready to use as supplied. Provided as a 5 mM stock solution in DMSO. Store at -20°C, stable for 5 freeze/thaw cycles.

5.4 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.

5.5 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.

5.6 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.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. 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.
2. 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.

Standard #	100 µM Fluorescence Standard (µL)	PTPase Assay Buffer (µL)	Final volume standard in well (µL)	Fluorescence Standard in well (pmol/well)
1	0	100	100	0
2	2	98	100	200
3	4	96	100	400
4	6	94	100	600
5	8	92	100	800
6	12	88	100	1200
7	16	84	100	1600
8	20	80	100	2000

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

We recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as general use cocktail.

7.1 Mammalian tissue or cells:

1. 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.
2. Homogenize mammalian soft tissues (~50 mg) or pelleted, pre-washed cells (~5 x 10^6 cells) in 500 µl ice-cold PTPase Assay Buffer (with DTT).
3. Incubate the homogenate on ice for 5 minutes and centrifuge at 10,000 x *g* and 4°C for 15 minutes. Collect the supernatant and keep on ice until use.

ΔNote: Tissue homogenates and cell lysates can also be aliquoted and stored at -80°C for future experiments.

ΔNote: Always prepare fresh PTPase Assay Buffer with DTT. Once prepared, keep buffer with DTT on ice and use within 4 hours.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Reaction wells set up:

- Standard wells = 80 µL standard dilutions.
- Sample wells = 2 -20 µL samples (adjust volume to 80 µL/well with PTPase Assay Buffer (with DTT)).
- Positive Control = 10 µL samples (adjust volume to 80 µL/well with PTPase Assay Buffer (with DTT)).

8.2 PTPase Reaction mixes:

1. Prepare 80 µL of the appropriate Reaction Mix for each reaction. Prepare a master mix to ensure consistency.

Component	Test Sample (µL)	+PTPase Inhibitor (µL)	Background Control (µL)	Positive Control (µL)
Sample	2 - 20	2 - 20	-	-
PTPase Positive Control	-	-	-	10
Suramin 2 mM solution	-	10	-	-
PTPase Assay Buffer (with DTT)	To 80 µL	To 80 µL	80	70

2. Preincubate the plate for 10 minutes 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 in Assay Buffer with DTT. 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).

3. 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.

ΔNote: Do not add PTPase Substrate solution to the standard curve wells.

8.3 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).

ΔNote: The standard curve wells may be read in endpoint mode (Ex/Em = 368/460 nm).

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. For the Fluorescence Standard curve, subtract the 0 pmol/well reading from all standard readings.
3. Plot the background subtracted values and calculate the slope.
4. 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: $\Delta F = RFU_2 – RFU_1$.
5. Calculate the specific fluorescence generated by PTPase activity (denoted by C_S) by subtracting the Suramin inhibitor control (ΔFI) from the corresponding test sample (ΔFS): $CS = \Delta FS – \Delta FI$.
6. 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.

$$\text{PTPase specific activity (A)} = \frac{B}{\Delta T * P}$$

Where:

A = PTPase specific activity in pmol/minute/mg = µU/mg

B = amount of product produced as calculated from the standard curve (pmol).

ΔT = the linear phase reaction time $t_2 – t_1$ (in minutes).

P = the amount of protein added to the sample well (in mg).

10. Typical Data

Data provided **for demonstration purposes only**.

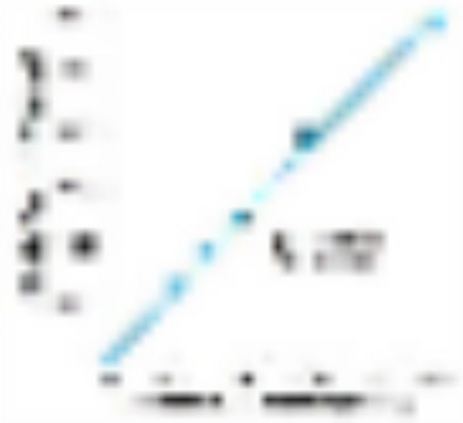

Figure 1. Fluorescence Standard curve. One mole of Fluorescence Standard corresponds to the metabolism of one mole of PTPase Substrate with release of one mole free inorganic phosphate.

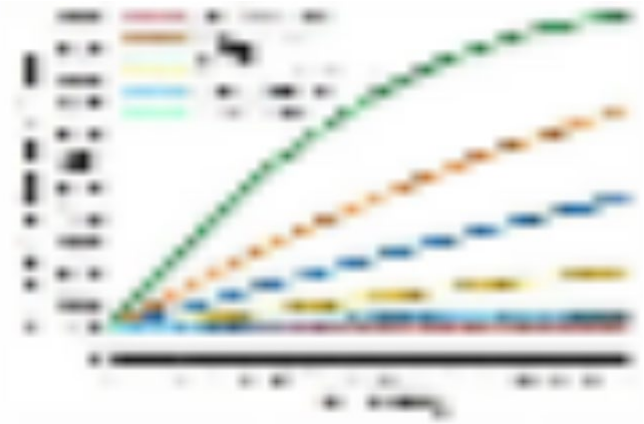

Figure 2. Reaction kinetics of PTPase Substrate metabolism in homogenates of human placenta (5 µg) and rat prefrontal cortex (10 µg) in the presence and absence of the PTPase inhibitor Suramin.

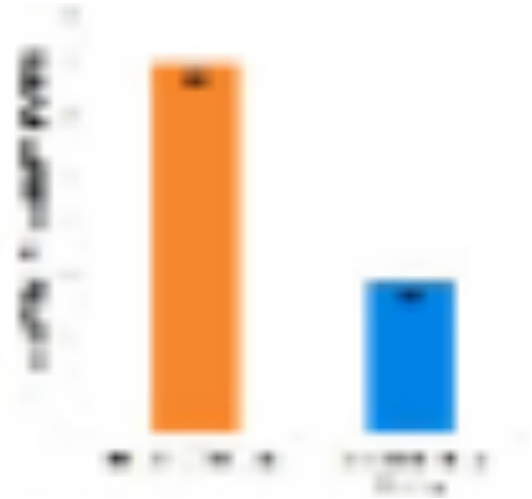

Figure 3. Quantification of PTPase activity in samples (mean ± SEM of 4 or more independent replicates).

11. Notes