Fluorometric Assay Kit for Measuring PTP1B Activity

CycLex Protein Tyrosine Phosphatase PTP1B Fluorometric Assay Kit

100 Assays

Cat# CY-1350

Intended Use

The CycLex Research product **Protein Tyrosine Phosphatase PTP1B Fluorometric Assay Kit** is a fluorometric and non-radioactive assay designed to measure the activity of protein tyrosine phosphatase, especially Protein Tyrosine Phosphatase 1B (PTP1B). This 96-well assay is useful for screening inhibitors and modulators of PTP1B activity in HTS. The kit includes all necessary components, including recombinant, human PTP1B (full length, residues 1-436 a.a.), for use in preinvestigational drug discovery assays.

This assay kit is for research use only and not for use in human, diagnostic, or therapeutic procedures.

Storage

- Upon receipt, store the kit at -70°C.
- Don't expose reagents to excessive light.
- **AVOID REPEATED FREEZE THAW CYCLES OF "③Recombinant PTP1B"!**

Introduction

The protein-tyrosine phosphatase PTP1B, a ubiquitous, non-transmembrane protein tyrosine phosphatase, is responsible for negatively regulating insulin signaling by dephosphorylating the phosphotyrosine residues of the insulin receptor kinase.

Elchebly et al. (1999) generated PTP1B-deficient mice by targeted disruption of the mouse homolog of the PTP1B gene. Mice were phenotypically and pathologically normal and had normal life span. In the fed state, homozygous mutant mice had slightly lower blood glucose concentrations, and half the circulating insulin concentrations, of wild type littermates. The enhanced insulin sensitivity of PTP1B-deficient mice was also evident in glucose- and insulin-tolerance tests. After insulin injection, deficient mice showed increased phosphorylation of the insulin receptor in liver and muscle tissue compared to wild type mice. On a high-fat diet, PTP1B-deficient mice were resistant to weight gain and remained insulin sensitive, while wild type mice rapidly gained weight and became insulin resistant. These results suggested a major role for PTP1B in modulation of insulin sensitivity and fuel metabolism, possibly involving PTP1B regulation of the leptin receptor pathway. Therefore it has been proposed that PTP1B is a potential therapeutic target for the treatment of type II diabetes and obesity.

Principle of the Assay

Measurement of PTP1B activity

The **Protein Tyrosine Phosphatase PTP1B Fluorometric Assay Kit** is based on an exclusive fluorescence substrate and development reagent combination. This homogenous assay kit is highly sensitive and convenient. This new method of measurement should dramatically raise the efficiency of inhibitor screening and biochemical analysis of these enzymes. The principle of the assay is as follows:

1. First, Fluoro-Phospho-Substrate, which comprises a unique PTP substrate containing a phospho group, is incubated with human PTP1B enzyme (full length, residues 1-436 a.a., expressed in E. coli).

2. Dephosphorylation of the substrate sensitizes the substrate so that, in the second step, treatment with the Development solution produces a fluorophore.

3. The fluorophore can be easily analyzed with a fluorescence plate reader or a fluorometer. The assay is suitable for high throughput screening applications.

Summary of Procedure

Two-Step Method

Mix 40 µL of Reaction Buffer and 5 µL of test compound in the wells
↓
Add 5 µL of recombinant PTP1B
↓ Incubate for 15 min at room temp.
Add 20 µL of Development Buffer and 5 µL of Development Reagent
↓ Incubate for 15 min at room temp.
Add 25 µL of Stop Solution
↓
Measure fluorescence at 510-530 nm emission / 482-502 nm excitation

One-Step Method

Mix 40 µL of Reaction Buffer containing Development Reagent and 5 µL of test compounds in the wells
↓
Add 5 µL of recombinant PTP1B
↓ Incubate for 10-20 min at room temp.
Add 25 µL of Stop Solution
↓
Measure fluorescence at 510-530 nm emission / 482-502 nm excitation

Note: The One-Step Method may provide lower sensitivity or higher background in the presence of high concentrations of Recombinant PTP1B as compared with the Two-Step Method.

Reminders for Kit Use

- Please use all reagents only after they are completely thawed and mixed.
- Please keep all reagents on ice until use.

Materials Provided

All samples and standards should be assayed in duplicate. The following components are supplied and are sufficient for one hundred assays.

Components of Kit

Components	Quantity	Storage
① **10X PTP Assay Buffer**	**700 µL x 1**	**Below -20°C**
② **10X Fluoro-Phospho-Substrate**	**500 µL x 1**	**Below -20°C**
③ **Recombinant PTP1B, Human (20 m units/µL)**	**500 µL x 1**	**-70°C**
④ **Development Buffer**	**1000 µL x 2**	**Below -20°C**
⑤ **Development Reagent**	**500 µL x 1**	**-70°C**
⑥ **Stop Solution**	**1300 µL x 2**	**-70°C**
⑦ **Phosphatase Inhibitor (400 mM Sodium Orthovanadate)**	**500 µL x 1**	**Below -20°C**
⑧ **10X Fluoro-Non-Phospho-Substrate**	**100 µL x 1**	**Below -20°C**
Instruction Manual	**1**	**Room temp.**

Materials Required but not Provided

- **Microtiter plate suitable for use with a fluorometric plate reader**
- **Fluorometric plate reader or microtiter plate fluorometer** capable of excitation at a wavelength in the range 482-502 nm and detection of emitted light in the range 510-530 nm.
- **Pipettors:** 2-20 µL, 20-200 µL and 200-1000 µL precision pipettors with disposable tips
- **Multi-channel pipette**
- **Microtiter plate shaker**
- **Distilled water** (DW) or equivalent high quality water
- **Microcentrifuge and tubes** for sample preparation
- **Reagent reservoirs**
- **Ice bucket** to keep reagents cold until use

Precautions and Recommendations

- Store the kit at -70°C.
- Do not expose reagents to excessive light.
- Do not use kit components beyond the indicated kit expiration date.
- Rinse all detergent residue from glassware.
- Use deionized water of the highest quality.
- Do not mix reagents from different kits.
- Do not mouth pipette or ingest any of the reagents.
- Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are

handled.

• Human samples may be contaminated with infectious agents. Do not ingest, expose to open wounds or breathe aerosols. Wear protective gloves and dispose of biological samples properly.

NOTE: THE FOLLOWING PROCEDURES ARE INTENDED ONLY AS A GUIDELINE. THE OPTIMAL EXPERIMENTAL CONDITIONS WILL VARY DEPENDING ON THE PARAMETERS BEING INVESTIGATED, AND MUST BE DETERMINED BY THE INDIVIDUAL USER.

Detailed Protocol

The **Protein Tyrosine Phosphatase PTP1B Fluorometric Assay Kit** can measure the enzyme activity of PTP1B by two different measuring methods, the Two-Step Method and the One-Step Method.

The Two-Step Method begins by initiating a reaction of recombinant PTP1B and the Fluoro-Phospho-Substrate, which is a substrate of PTP1B, to remove a phosphate group from substrate. The second step involves addition of the Development Solution and the Stop Solution.

In the One-Step Method, the reaction is initiated by mixing the Fluoro-Phospho-Substrate and recombinant PTP1B in the presence of the Development Reagent. The researcher may then stop the reaction by adding Stop Solution and measure the fluorescence intensity. Alternatively, it is possible to measure fluorescence intensity at regular intervals after the reaction is initiated and to determine reaction kinetics without stopping the reaction.

Caution and Significance

• "**③Recombinant PTP1B**" and "**⑤Development Reagent**" should be stored at -70°C. **AVOID REPEATED FREEZE THAW CYCLES OF "③Recombinant PTP1B"!** Making aliquot of "**③ Recombinant PTP1B**" is recommended. Thaw the other reagents at room temperature and keep all reagents on ice until use. Use them only after they are completely thawed and mixed.

• All samples and standards should be assayed in duplicate.

• Use of a microtiter plate shaker is recommended for complete mixing.

• In order to estimate the inhibitory effect on PTP1B activity by the test compounds correctly, it is necessary to conduct the control experiment of "**Solvent control**" at least once for every experiment and "**Inhibitor control**" at least once for the first experiment, in addition to "**Test sample**" as indicated in the following table (below). When test chemicals cause an inhibitory effect on PTP1B activity, the level of increase of fluorescence intensity is weakened as compared with "**Solvent control**". The increase in fluorescence intensity is not observed in "**Inhibitor control**".

• If the test compounds or samples have an inhibitory effect on the development reaction, the final fluorescence intensity will not increase. Please use Fluoro-Non-phospho-Substrate instead of Fluoro-phospho-Substrate as "**Development control**", and conduct a control experiment that does not add PTP1B.

• Conversely, if test compounds or samples have a stimulatory effect on the development reaction, resulting in an increase of fluorescence intensity independent of the phosphatase reaction, the assays cannot be evaluated correctly. To check this possibility, do reactions with or without addition of Recombinant PTP1B in the presence of Fluoro-phospho-Substrate. If a test compound has a

stimulatory effect on the development reaction, fluorescence intensity will increase even if PTP1B is not added to the reaction.

• If the test compounds or samples themselves emit fluorescence at excitation wavelength: 482-502 nm and fluorescence wavelength: 510-530 nm, the test assay cannot be evaluated correctly.

Two-Step Method

1. Following Table.1 below, first, add **"Distilled water"**, **"①10X PTP Assay Buffer"** and **"②10X Fluoro-Phospho-Substrate"** to microtiter plate wells. Second, add **"Test Compound"** or **"Solvent of Test Compounds"** or **"⑦Phosphatase Inhibitor"** to each well of the microtiter plate and mix well.

Table.1: Reaction mixture of Two-Step Method

Assay reagents	Test Sample	Solvent Control	Inhibitor Control	No Enzyme Control	Development Control
Distilled water	**30 µL**	**30 µL**	**30 µL**	**30 µL**	**30 µL**
①10X PTP Assay Buffer	**5 µL**	**5 µL**	**5 µL**	**5 µL**	**5 µL**
②10X Fluoro-Phospho-Substrate	**5 µL**	**5 µL**	**5 µL**	**5 µL**	**-**
⑧10X Fluoro-Non-Phospho-Substrate	**-**	**-**	**-**	**-**	**5 µL**
Test Compound	**5 µL**	**-**	**-**	**-**	**5 µL**
Solvent of Test Compounds	**-**	**5 µL**	**-**	**5 µL**	**-**
⑦Phosphatase Inhibitor	**-**	**-**	**5 µL**	**-**	**-**
③Recombinant PTP1B (20 m units/µL)	**5 µL**	**5 µL**	**5 µL**	**-**	**-**
Distilled water	**-**	**-**	**-**	**5 µL**	**5 µL**
Total Volume of the mixture	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**

2. Initiate reactions by adding **5 µL of "③Recombinant PTP1B"** or distilled water to each well and mixing thoroughly at room temperature.

3. Incubate for 15 min or desired length of time at room temperature.

4. Add **20 µL of "④Development Buffer"** and **5 µL of "⑤Development Reagent"** to each well of the microtiter plate, and mix thoroughly.

5. Incubate approximately for 15 min at room temperature.

6. Add **25 µL of "⑥Stop Solution"** to each well of the microtiter plate, and mix thoroughly.

7. Measure fluorescence intensity using a microtiter plate fluorometer with excitation at 482-502 nm and emission at 510-530 nm.

8. The efficacy of the Test compound is the difference in fluorescence intensity between "Solvent control" and "Test sample".

Note: If necessary, it is possible to store the microtiter plate after adding **"⑥Stop Solution"** for a few hours at room temperature or up to 24 hours at 4°C. The microtiter plate must be sealed to prevent evaporation and kept from excessive light.

One-Step Method

1. Following Table.2 below, first, add **"Distilled water"**, **"①10X PTP Assay Buffer"** and **"②10X Fluoro-Phospho-Substrate"** to microtiter plate wells. Second, add **"Test Compound"** or **"Solvent of Test Compounds"** or **"⑦Phosphatase Inhibitor"** to each well of the microtiter plate. Finally, just before initiation of the reaction with PTP1B, add **5 µL of "⑤Development Reagent"** and mix well.

Table.2: Reaction mixture of One-Step Method

Assay reagents	Test Sample	Solvent Control	Inhibitor Control	No Enzyme Control	Development Control
Distilled water	25 µL	25 µL	25 µL	25 µL	25 µL
①10X PTP Assay Buffer	5 µL	5 µL	5 µL	5 µL	5 µL
②10X Fluoro-Phospho-Substrate	5 µL	5 µL	5 µL	5 µL	-
⑧10X Fluoro-Non-Phospho-Substrate	-	-	-	-	5 µL
Test Compound	5 µL	-	-	-	5 µL
Solvent of Test Compounds	-	5 µL	-	5 µL	-
⑦Phosphatase Inhibitor	-	-	5 µL	-	-
⑤Development Reagent	5 µL	5 µL	5 µL	5 µL	5 µL
③Recombinant PTP1B (20 m units/µL)	5 µL	5 µL	5 µL	-	-
Distilled water	-	-	-	5 µL	5 µL
Total Volume of the mixture	50 µL	50 µL	50 µL	50 µL	50 µL

2. Initiate reactions by adding **5 µL of "③Recombinant PTP1B"** or distilled water to each well and mixing thoroughly at room temperature.

3. Add **25 µL of "⑥Stop Solution"** to each well at appropriate time, and measure fluorescence intensity using a microtiter plate fluorometer with excitation at 482-502 nm and emission at 510-530 nm.

4. The efficacy of the Test compound is the difference in fluorescence intensity between "Solvent control" and "Test sample".

Alternate procedure

3'. Read fluorescence intensity for several minutes using a microtiter plate fluorometer with excitation at 482-502 nm and emission at 510-530 nm.

4'. Measure and calculate the rate of reaction while the reaction velocity remains constant.

Note-1: In some cases, the **One-Step Method** may cause higher backgrounds and lower sensitivity as compared with the **Two-Step Method**. In many cases, there will be no measurable difference between the two methods. Choose the most suitable method for your particular experiment.

Note-2: If necessary, it is possible to store the microtiter plate after adding **"⑥Stop Solution"** for a few hours at room temperature or up to 24 hours at 4°C. The microtiter plate must be sealed to prevent evaporation and kept from excessive light.

Evaluation of Results

Analysis of Inhibitor Effect

% Intensity

1. Run reactions with test compounds and solvent as described in the **Detailed Protocol**.
2. Subtract fluorescence intensity of "No Enzyme Control" from all experimental samples (Test Samples and Solvent Control).
3. Calculate the % Intensity:

$$\% \text{ Intensity} = \frac{\text{Fluorescence Intensity of Test Sample}}{\text{Fluorescence Intensity of Solvent Control}} \text{ X } 100$$

Note: This % Intensity is a rough value of enzyme activity or inhibition. For greater accuracy, plot a standard curve of PTP1B for each new set of reactions and estimate the % Activity (see below).

Fig.1 PTP1B Inhibition Curve by Sodium Orthovanadate using the Two-Step Method

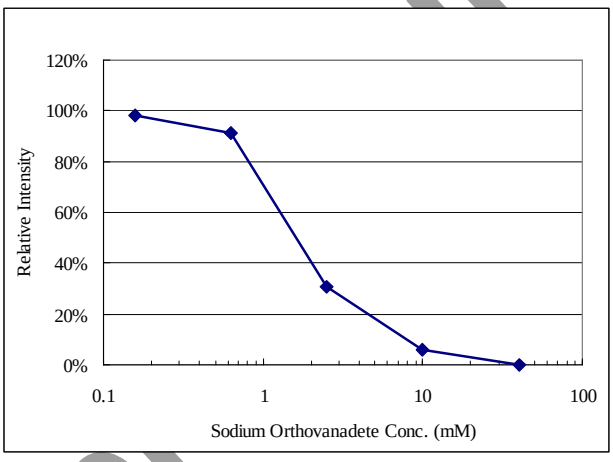
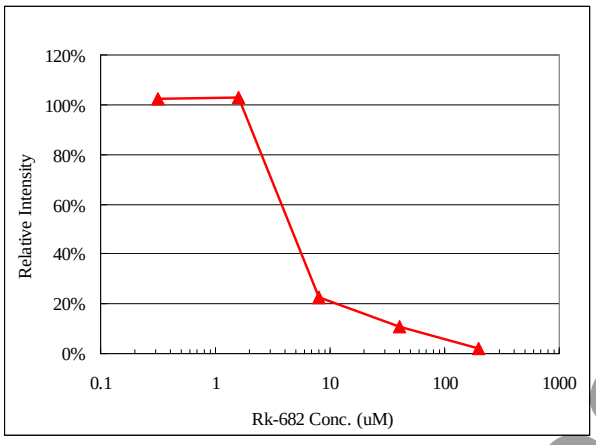


Fig.2 PTP1B Inhibition Curve by RK-682 using the Two-Step Method



Analysis of Enzyme Activity

PTP1B Standard Curve and % Activity

1. Dilute the ①10X PTP Assay buffer 1:9 with distilled water to make 1X PTP Assay Buffer.
2. Make serial dilutions of PTP1B with 1X PTP Assay Buffer (ex. 100%, 75%, 50%, 25% 10% and 0%).
3. Run reactions with solvent and serial dilutions of PTP1B as described in the **Detailed Protocol**.
4. Plot standard curve data (dose dependent curve data) as fluorescence intensity at 510-530 nm versus dose of PTP1B (unit/assay)
5. Obtain a line-fit to the data using appropriate calculations.
6. Use the slope and Y-intercept to calculate the amount of PTP1B activity for the experimental data.

Fig.3 Dose Dependency of Recombinant PTP1B using the Two-Step Method

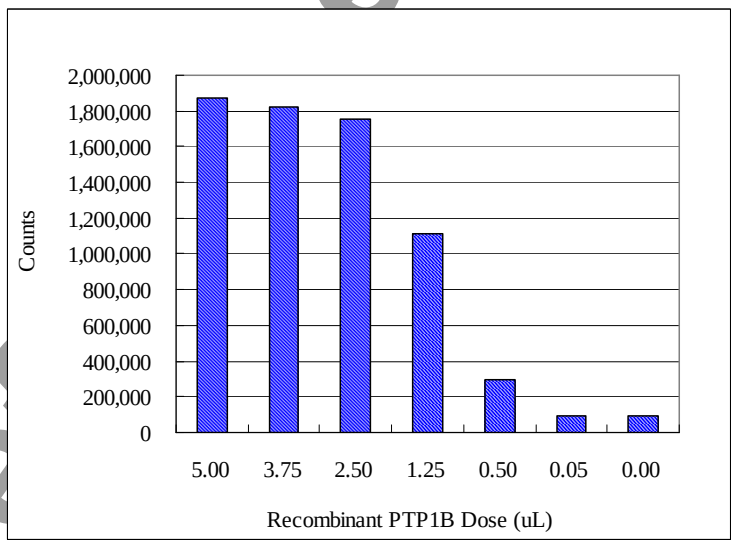
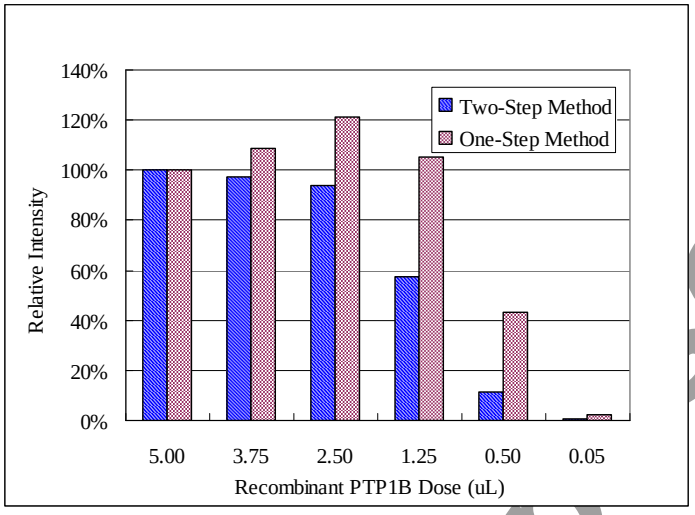


Fig.4 Comparison between Two-Step Method and One-Step Method in Dose Dependency of Recombinant PTP1B



Analysis of Kinetics

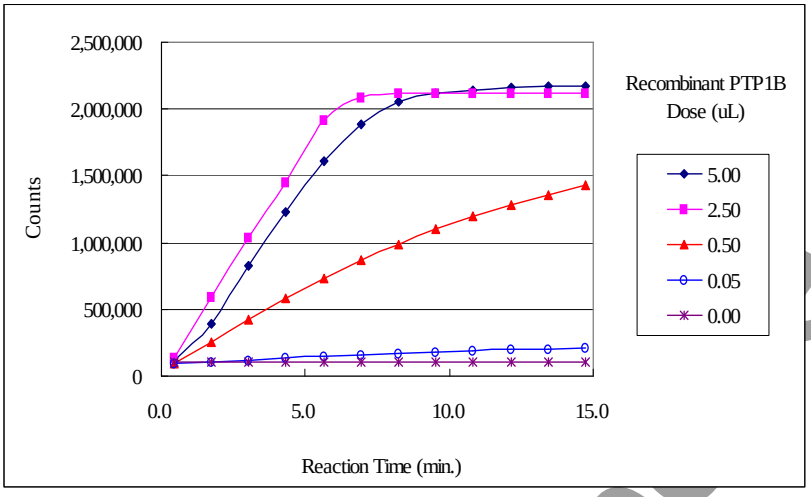
Time Course Curve (Only for One-Step Method)

1. Run reactions as described in the **Detailed Protocol**.
2. Subtract fluorescence intensity at the 0 time from all reaction time points.
3. Plot fluorescence intensity at 510-530 nm versus reaction time.
4. Determine the reaction time range in which the increase in fluorescence intensity at 510-530 nm is linear.
5. Calculate activity:

$$\text{Activity (reaction velocity)} = \frac{\text{Fluorescence Intensity of Test Sample}}{\text{Reaction time (min.)}}$$

Note: Usually, the linear range is from 0 to 10 min. This value is variable depending on reaction conditions and storage/handling of the PTP1B. Decreasing the amount of PTP1B in the assay may help to lengthen the time range.

Fig.5 Time Course Curve of Recombinant PTP1B using the One-Step Method



Troubleshooting

1. The PTP1B positive control should be run in duplicate using the protocol described in the **Detailed Protocol**. Incubation times or temperatures significantly different from those specified may give erroneous results.

2. The reaction curve is nearly a straight line if the kinetics of the assay is of the first order. Variations in the protocol can lead to non-linearity of the curve, as can assay kinetics of other than first order. For a non-linear curve, point to point or quadratic curve fit methods should be used.

3. Poor duplicates, accompanied by elevated values for wells containing no sample, indicate inaccurate dispensing of assay reagents. If all instructions in the **Detailed Protocol** were followed accurately, such results indicate a need for multi-channel pipette maintenance.

Reagent Stability

All of the reagents included in the **Protein Tyrosine Phosphatase PTP1B Fluorometric Assay Kit** have been tested for stability. Reagents should not be used beyond the stated expiration date. Upon receipt, all the kit should be stored at -70°C. After use, return the kit to -70°C as soon as possible.

For research use only, not for use in human, diagnostic or therapeutic procedures

References

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Related Products

*** CycLex Protein Tyrosine Phosphatase PTP1B Fluorometric Assay Kit: Cat# CY-1350**
*** CycLex Protein Tyrosine Phosphatase TC-PTP Fluorometric Assay Kit: Cat# CY-1351**
*** CycLex Protein Phosphatase Cdc25A Fluorometric Assay Kit: Cat# CY-1352**
*** CycLex Protein Phosphatase Cdc25B Fluorometric Assay Kit: Cat# CY-1353**
*** CycLex Protein Phosphatase Cdc25C Fluorometric Assay Kit: Cat# CY-1354**
*** CycLex Protein Phosphatase Cdc25 Combo Fluorometric Assay Kit: Cat# CY-1355**
*** CycLex Protein Phosphatase Cdi1/KAP Fluorometric Assay Kit: Cat# CY-1356**
*** CycLex Protein Phosphatase LMW-PTP/ACP1 Fluorometric Assay Kit: Cat# CY-1358**
*** CycLex Protein Phosphatase DUSP1/MKP-1 Fluorometric Assay Kit: Cat# CY-1373**

*** Protein Tyrosine Phosphatase PTPRA 1st Catalytic Domain: Cat# CY-E1301**
*** Protein Tyrosine Phosphatase PTPRA 2nd Catalytic Domain: Cat# CY-E1302**
*** Protein Tyrosine Phosphatase PTPRD 2nd Catalytic Domain: Cat# CY-E1307**
*** Protein Tyrosine Phosphatase PTPRE 1st Catalytic Domain: Cat# CY-E1308**
*** Protein Tyrosine Phosphatase PTPRF 1st Catalytic Domain: Cat# CY-E1310**
*** Protein Tyrosine Phosphatase PTPRK 1st Catalytic Domain: Cat# CY-E1316**
*** Protein Tyrosine Phosphatase PTPRQ: Cat# CY-E1323**
*** Protein Tyrosine Phosphatase PTP4A2: Cat# CY-E1341**
*** Recombinant Cdc25A (Catalytic domain): Cat# CY-E1352**
*** Recombinant Cdc25B (Catalytic domain): Cat# CY-E1353**
*** Recombinant Cdc25C (Catalytic domain): Cat# CY-E1354**
*** Recombinant Cdi1/KAP: Cat# CY-E1356**
*** Protein Phosphatase PP5: Cat# CY-E1359**
*** Protein Tyrosine Phosphatase PTPN3/PTPH1: Cat# CY-E1360**
*** Protein Tyrosine Phosphatase PTPN6/SHP-1: Cat# CY-E1363**

*** Protein Tyrosine Phosphatase PTPN7/HePTP: Cat# CY-E1364**
*** Protein Tyrosine Phosphatase PTPN8/PTPN22: Cat# CY-E1365**
*** Protein Tyrosine Phosphatase PTPN9/MEG2: Cat# CY-E1366**
*** Protein Tyrosine Phosphatase PTPN11/SHP-2: Cat# CY-E1367**
*** Protein Tyrosine Phosphatase PTPN12/PTP-PEST: Cat# CY-E1368**
*** Protein Tyrosine Phosphatase PTPN13/FAP-1: Cat# CY-E1369**
*** Protein Tyrosine Phosphatase PTPN14/PEZ: Cat# CY-E1370**
*** Protein Tyrosine Phosphatase PTPN21/PTPD1: Cat# CY-E1372**
*** Protein Phosphatase DUSP1/MKP-1: Cat# CY-E1373**

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