Fluorometric Assay Kit for Measuring LMW-PTP/ACP1 Phosphatase Activity

CycLex Protein Phosphatase LMW-PTP/ACP1 Fluorometric Assay Kit

100 Assays

Cat# CY-1358

Intended Use

The MBL Research product **Protein Phosphatase LMW-PTP/ACP1 Fluorometric Assay Kit** is a fluorometric and non-radioactive assay designed to measure the activity of LMW-PTP/ACP1 protein phosphatase. This 96-well assay is useful for screening inhibitors and modulators of LMW-PTP/ACP1 activity in HTS. The kit includes all necessary components, including recombinant, human full length LMW-PTP/ACP1, 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 "#3.Recombinant LMW-PTP/ACP1"!**

Introduction

Protein tyrosine phosphorylation plays an essential role in the regulation of many cellular processes, including cellular proliferation, differentiation, migration and tumorigenic transformation. The phosphorylation of proteins on tyrosine is catalyzed by numerous protein tyrosine kinases, and is rapidly and reversibly dephosphorylated by Protein tyrosine phosphatases (PTPases).

The low molecular weight protein tyrosine phosphatase (LMW-PTP) is an 18-kDa cytosolic enzyme, also known as acidic protein phosphatase 1 (ACP1). LMW-PTP/ACP1 is specific for phosphotyrosine in peptides and proteins, but the enzyme shares very limited sequence homology with other PTPases.

Although LMW-PTP/ACP1 has been showed as negative regulator of insulin- and platelet-derived growth factor (PDGF)-mediated mitotic and metabolic signaling, LMW-PTP/ACP1 is frequently overexpressed in transformed cell. Recent studies suggested that entopic overexpression of LMW-PTP/ACP1 is sufficient to confer transformation in epithelial cells and its oncogenic activities required EphA2. LMW-PTP/ACP1 negatively regulates EphA2 receptor tyrosine kinase. LMW-PTP/ACP1 is a positive regulator of both tumor onset and development through ephrin-EphA2 signaling process, and it is a potential target of anticancer drug development.

Principle of the Assay

The **Protein Phosphatase LMW-PTP/ACP1 Fluorometric Assay Kit** is based on an exclusive fluorescence substrate, OMFP (3-o-methylfluorescein phosphate). This homogenous assay kit is sensitive and convenient. This method of measurement should raise the efficiency of inhibitor screening and biochemical analysis of this enzyme.

Summary of Procedure

Mix 40 µL of Assay Mixture and 5 µL of test compound in the wells

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Add 5 µL of Recombinant LMW-PTP/ACP1

↓ Incubate for 15 minutes at room temp.

Add 25 µL of Stop Solution

↓

Measure fluorescence at 510-540 nm emission / 482-502 nm excitation

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 LMW-PTP/ACP1 Assay Buffer	**600 µL x 1**	**Below -20°C**
#2. 10X OMFP	**550 µL x 1**	**Below -20°C**
#3. Recombinant LMW-PTP/ACP1 (Human)*	**500 µL x 1**	**-70°C**
#4. 100X Phosphatase Inhibitor (10 mM Na_3VO_4)	**100 µL x 1**	**Below -20°C**
#5. Stop Solution	**1,300 µL x 2**	**Below -20°C**
Instruction Manual	**1**	**Room temp.**

* "#3. Recombinant LMW-PTP/ACP1" is human full length with N-terminal GST-Tag. The GenBank Accession No. is BC106011.

Materials Required but not Provided

- **Microtiter plate suitable for use with a fluorometric plate reader**
- **Fluorometric plate reader or microtiter plate fluorometer:** Use a fluorescence microplate reader equipped with appropriate filters. OMFP has excitation/emission maxima of approximately 485/525 nm. We have found that standard filters for blue-fluorescent dyes (e.g., excitation = 485 ±12.5 nm, emission = 525 ± 20 nm) can be used to detect OMFP.
- **Pipettors:** 2-20 µL, 20-200 µL and 200-1,000 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

- Upon receipt, 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.

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

Preparation of Reagents

Thaw the reagents at room temperature except **"#3. Recombinant LMW-PTP/ACP1"** and keep all reagents including **"#3. Recombinant LMW-PTP/ACP1"** on ice until use. **AVOID REPEATED FREEZE THAW CYCLES OF "#3. Recombinant LMW-PTP/ACP1"!** Making aliquot of **"#3. Recombinant LMW-PTP/ACP1"** is recommended. Use them only after they are completely thawed and mixed.

1. Prepare **10X Phosphatase Inhibitor** by adding 5 µL of **"#4. 100X Phosphatase Inhibitor"** to 45 µL of distilled (deionized) water. Mix well.

   *Discard any unused **10X Phosphatase Inhibitor** after use.*

2. Prepare **Assay Mixture** by adding 5 µL of **"#1. 10X Assay Buffer"** and 5 µL of **"#2. 10X OMFP"** to 30 µL of distilled (deionized) water per one assay. Mix well.

Assay Mixture

Assay reagents	1 assay	8 assays	16 assays	32 assays	48 assays
Distilled water	**30 µL**	**240 µL**	**480 µL**	**960 µL**	**1,440 µL**
#1. 10X LMW-PTP/ACP1 Assay Buffer	**5 µL**	**40 µL**	**80 µL**	**160 µL**	**240 µL**
#2. 10X OMFP	**5 µL**	**40 µL**	**80 µL**	**160 µL**	**240 µL**
Total volume of Assay Mixture	**40 µL**	**320 µL**	**640 µL**	**1,240 µL**	**1,920 µL**

Assay Procedure

In order to estimate the inhibitory effect on LMW-PTP/ACP1 activity by the test compounds correctly, it is necessary to conduct the control experiment of **"Vehicle 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 Table.1 (below). When test chemicals cause an inhibitory effect on LMW-PTP/ACP1 activity, the level of increase of fluorescence intensity is weakened as compared with **"Vehicle Control"**. The increase in fluorescence intensity is not observed in **"Inhibitor Control"**.

1. Following Table.1 below, first, add **"Assay Mixture"** to microtiter plate wells. Second, add **"Test Compound"** or **"Vehicle of Test Compound"** or **"10X Phosphatase Inhibitor"** to each well of the microtiter plate and mix well.

Table.1: Reaction mixture

Assay reagents	Test Sample	Vehicle Control	Inhibitor Control	No Enzyme Control
Assay Mixture	**40 µL**	**40 µL**	**40 µL**	**40 µL**
Test Compound **Vehicle of Test Compound** **10X Phosphatase Inhibitor***	**5 µL** **-** **-**	**-** **5 µL** **-**	**-** **-** **5 µL**	**-** **5 µL** **-**
#3. Recombinant LMW-PTP/ACP1 **Distilled water**	**5 µL** **-**	**5 µL** **-**	**5 µL** **-**	**-** **5 µL**
Total Volume of the Reaction mixture	**50 µL**	**50 µL**	**50 µL**	**50 µL**

*10X Phosphatase Inhibitor (1mM Na_3VO_4): See the section "Preparation of Reagents" above.

2. Initiate reactions by adding **5 µL** of **"#3. Recombinant LMW-PTP/ACP1"** or distilled water to each well and mixing thoroughly at room temperature.

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

4. Add **25 µL** of **"#5. Stop Solution"** to each well of the microtiter plate, and mix thoroughly.

5. Measure fluorescence intensity using a microtiter plate fluorometer with excitation at 482-502 nm and emission at 510-540 nm.

6. The efficacy of the Test compound is the difference in fluorescence intensity between **"Vehicle Control"** and **"Test Sample"**.

Note: If necessary, it is possible to store the microtiter plate after adding **"#5. Stop Solution"** for a few hours at 4°C. The microtiter plate must be sealed to prevent evaporation and kept from excessive light.

Alternate procedure

1. Following Table.1 above, first, add **"Assay Mixture"** to microtiter plate wells. Second, add **"Test Compound"** or **"Vehicle of Test Compound"** or **"10X Phosphatase Inhibitor"** to each well of the microtiter plate and mix well.

2. Initiate reactions by adding **5 µL** of **"#3. Recombinant LMW-PTP/ACP1"** or distilled water to each well and mixing thoroughly at room temperature.

3. Read fluorescence intensity for 20 to 30 minutes at 1 to 2 minutes intervals using microtiter plate fluorometer with excitation at 482-502 nm and emission at 510-540 nm.

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

Caution and Significance

• All samples and "Recombinant LMW-PTP/ACP1" should be assayed in duplicate.

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

• If the test compounds or samples themselves emit fluorescence at excitation wavelength: 482-502 nm and fluorescence wavelength: 510-540 nm, the test assay cannot be evaluated correctly.

Evaluation of Results

Analysis of Inhibitor Effect

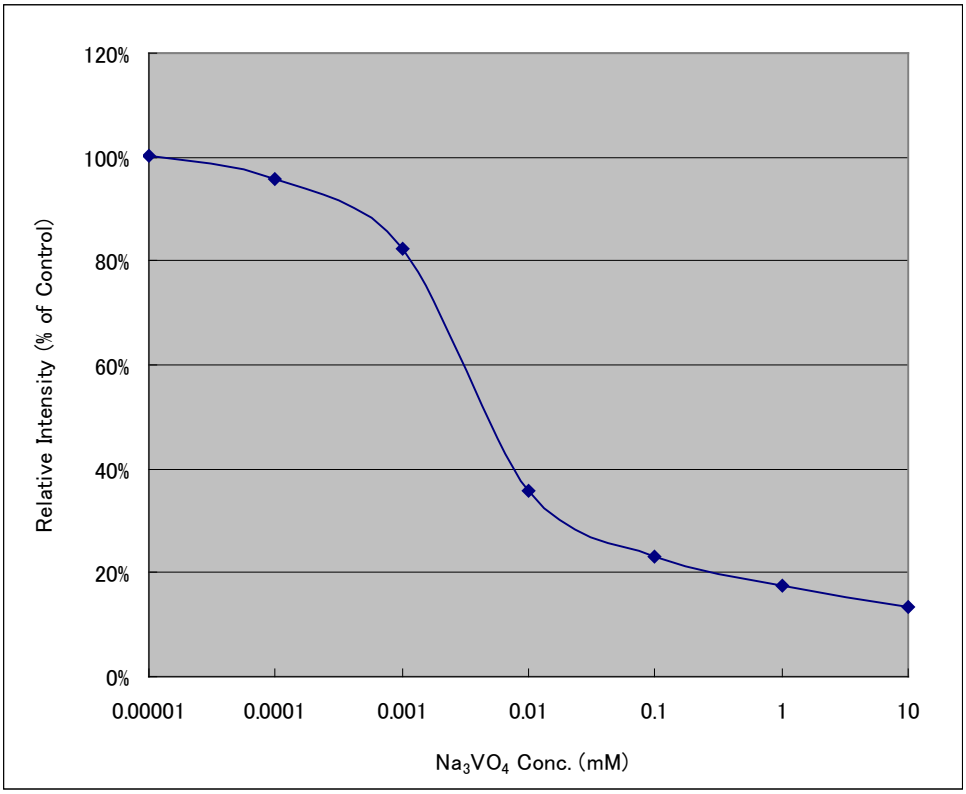
% Intensity

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

$$\%\ \text{Intensity} = \frac{\text{Fluorescence Intensity of Test Sample}}{\text{Fluorescence Intensity of Vehicle Control}} \times 100$$

Note: This % Intensity is a rough value of enzyme activity or inhibition. For greater accuracy, plot a standard curve of LMW-PTP/ACP1 for each new set of reactions and estimate the % Activity (see below).

Fig.1 LMW-PTP/ACP1 Inhibition Curve by Na_3VO_4 (SOV; Sodium orthovanadate)

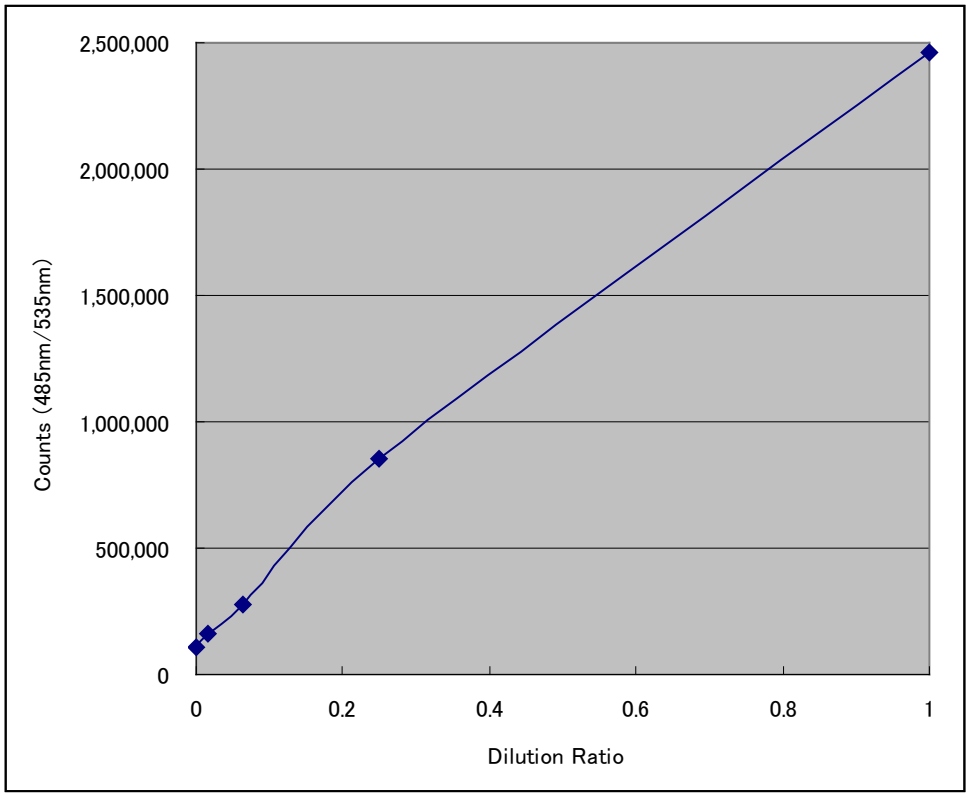


Analysis of Enzyme Activity

LMW-PTP/ACP1 Standard Curve and % Activity

1. Dilute "#1. 10X LMW-PTP/ACP1 Assay Buffer" 1:10 with distilled water to make 1X Assay Buffer.
2. Make serial dilutions of "#3. Recombinant LMW-PTP/ACP1" with 1X Assay Buffer (e.g. 100%, 25%, 6.25%, 1.56% and 0%).
3. Run reactions with vehicle and serial dilutions of Recombinant LMW-PTP/ACP1 as described in the Detailed Protocol.
4. Plot standard curve data (dose dependent curve data) as fluorescence intensity at 510-540 nm versus dose of LMW-PTP/ACP1.
5. Obtain a line-fit to the data using appropriate calculations.
6. Use the slope and Y-intercept to calculate the amount of LMW-PTP/ACP1 activity for the experimental data.

Fig.2 Dose Dependency of Recombinant LMW-PTP/ACP1



Analysis of Kinetics

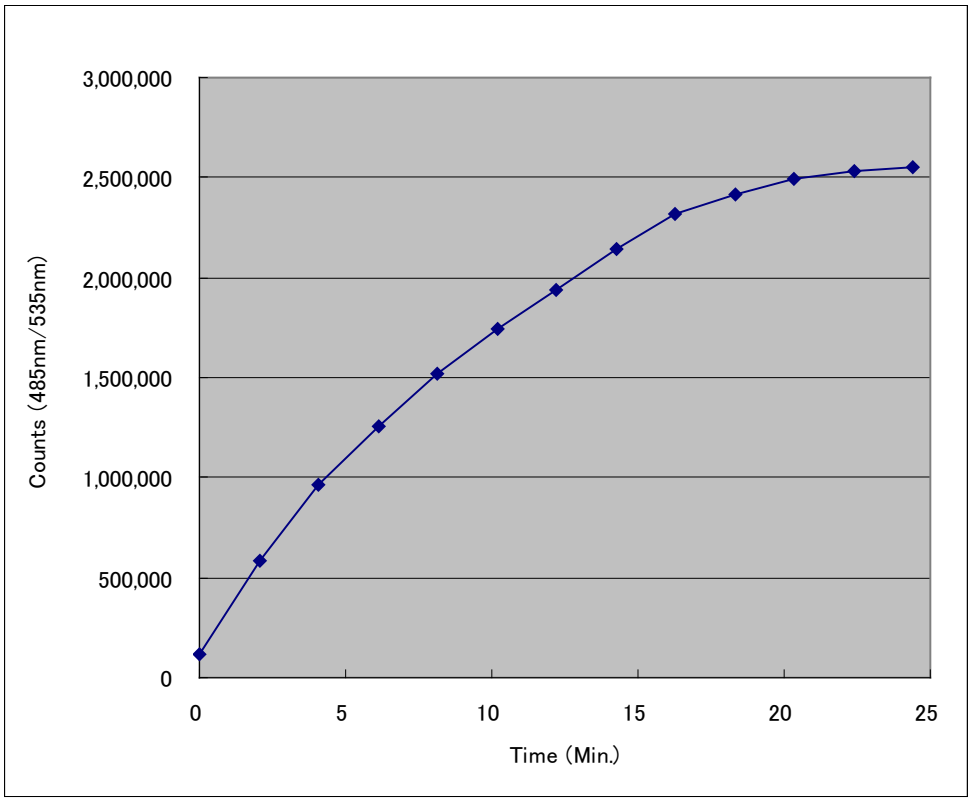
Time Course Curve

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 510-540 nm versus reaction time.
4. Determine the reaction time range in which the increase in fluorescence intensity at 510-540 nm is linear.
5. Calculate activity:

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

Note: Usually, the linear range is from 0 to 30 minutes. This value is variable depending on reaction conditions and storage/handling of the Recombinant LMW-PTP/ACP1. Decreasing the amount of Recombinant LMW-PTP/ACP1 in the assay may help to lengthen the time range.

Fig.3 Time Course Curve of Recombinant LMW-PTP/ACP1



Troubleshooting

1. All samples and controls should be assayed 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 Phosphatase LMW-PTP/ACP1 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.

References

1. Dissing J, Johnsen AH, Sensabaugh GF. Human red cell acid phosphatase (ACP1). The amino acid sequence of the two isozymes Bf and Bs encoded by the ACP1*B allele. J Biol Chem. 1991 Nov 5; 266(31):20619-25.

2. Wo YY, McCormack AL, Shabanowitz J, Hunt DF, Davis JP, Mitchell GL, Van Etten RL. Sequencing, cloning, and expression of human red cell-type acid phosphatase, a cytoplasmic phosphotyrosyl protein phosphatase. J Biol Chem. 1992 May 25; 267(15):10856-65.

3. Chiarugi P, Cirri P, Raugei G, Manao G, Taddei L, Ramponi G. Low M(r) phosphotyrosine protein phosphatase interacts with the PDGF receptor directly via its catalytic site. Biochem Biophys Res Commun. 1996 Feb 6; 219(1):21-5.

4. Chiarugi P, Cirri P, Marra F, Raugei G, Camici G, Manao G, Ramponi G. LMW-PTP is a negative regulator of insulin-mediated mitotic and metabolic signalling. Biochem Biophys Res Commun. 1997 Sep 18; 238(2):676-82.

5. Fiaschi T, Chiarugi P, Buricchi F, Giannoni E, Taddei ML, Talini D, Cozzi G, Zecchi-Orlandini S, Raugei G, Ramponi G. Low molecular weight protein-tyrosine phosphatase is involved in growth inhibition during cell differentiation. J Biol Chem. 2001 Dec 28; 276(52):49156-63.

6. Kikawa KD, Vidale DR, Van Etten RL, Kinch MS. Regulation of the EphA2 kinase by the low molecular weight tyrosine phosphatase induces transformation. J Biol Chem. 2002 Oct 18; 277(42):39274-9.

7. Chiarugi P, Taddei ML, Schiavone N, Papucci L, Giannoni E, Fiaschi T, Capaccioli S, Raugei G, Ramponi G. LMW-PTP is a positive regulator of tumor onset and growth. Oncogene. 2004 May 13; 23(22):3905-14.

8. Malentacchi F, Marzocchini R, Gelmini S, Orlando C, Serio M, Ramponi G, Raugei G. Up-regulated expression of low molecular weight protein tyrosine phosphatases in different human cancers. Biochem Biophys Res Commun. 2005 Sep 2; 334(3):875-83.

9. Parri M, Buricchi F, Taddei ML, Giannoni E, Raugei G, Ramponi G, Chiarugi P. EphrinA1 repulsive response is regulated by an EphA2 tyrosine phosphatase. J Biol Chem. 2005 Oct 7; 280(40):34008-18.

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