Protein Phosphatase LMW-PTP/ACP1 Fluorometric (Human) Assay Kit

Cat. No.: Kit-0737
Lot. No. (See product label)

Product Information

Product Overview
Protein Phosphatase LMW-PTP/ACP1 Fluorometric (Human) Assay Kit is a fluorometric and non-radioactive assay designed to measure the activity of LMW-PTP/ACP1 protein phosphatase.

Description
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 ectopic 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.

Applications
The 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.

Target Species
Human

Kit Components
10X Assay Buffer 600 µL x 1 Less than -20°C
10X OMFP 550 µL x 1 Less than -20°C
Recombinant LMW-PTP/ACP1 (20 m units/µL)* 500 µL x 1 -70°C
100X Phosphatase Inhibitor : 100 mM Na3VO4 in DW 100 µL x 1 Less than -20°C
Stop Solution 1,300 µL x 2 Less than -20°C
Instruction Manual 1 Room temp.*“Recombinant LMW-PTP/ACP1” is human full length with N-terminal GST-Tag. The GenBank Accession No. is BC~10^6011.

Materials Required but Not Supplied
* 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 200-1000 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

Usage
For research use only (RUO)

Storage
· Upon receipt, store the kit at –70°C
· Do not expose reagents to excessive light.
· AVOID REPEATED FREEZE THAW CYCLES OF "Recombinant LMW-PTP/ACP1"!
**Preparation**

Thaw the reagents at room temperature except "Recombinant LMW-PTP/ACP1" and keep all reagents including "Recombinant LMW-PTP/ACP1" on ice until use. AVOID REPEATED FREEZE THAW CYCLES OF "Recombinant LMW-PTP/ACP1"! Making aliquot of "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 the 100X Phosphatase Inhibitor (provided) 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 the 10X Assay Buffer (provided) and 5 µL of the 10X OMFP (provided) to 30 µL of distilled (deionized) water per one assay. Mix well.

**Assay Protocol**

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

<table>
<thead>
<tr>
<th>Table 1: Reaction mixture</th>
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</thead>
<tbody>
<tr>
<td><strong>Assay reagents</strong></td>
</tr>
<tr>
<td>Test Sample</td>
</tr>
<tr>
<td>Vehicle Control</td>
</tr>
<tr>
<td>Inhibitor Control</td>
</tr>
<tr>
<td>No Enzyme Control</td>
</tr>
<tr>
<td><strong>Vehicle of Test Compounds</strong></td>
</tr>
<tr>
<td><strong>10X Phosphatase Inhibitor</strong></td>
</tr>
<tr>
<td><strong>Recombinant LMW-PTP/ACP1 (20 m units/µL)</strong></td>
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<tr>
<td><strong>Total Volume of the Reaction mixture</strong></td>
</tr>
<tr>
<td>50 µL 50 µL 50 µL 50 µL 50 µL 50 µL</td>
</tr>
<tr>
<td>Na3VO4: See section Preparation of Reagents</td>
</tr>
</tbody>
</table>

2) Initiate reactions by adding 5 µL of "Recombinant LMW-PTP/ACP1" 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 25 µL of "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 "Stop Solution" for a few hours at 4°C. The microtiter plate must be sealed to prevent evaporation and kept from excessive light.

**Analysis**

Analysis of Inhibitor Effect #1

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:
   \[
   \text{% Intensity} = \left( \frac{\text{Fluorescence Intensity of Test Sample}}{\text{Fluorescence Intensity of Vehicle Control}} \right) \times 100
   \]
   *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. Analysis of Inhibitor Effect #2

LMW-PTP/ACP1 Standard Curve and % Activity

1. Dilute the 10X Assay buffer 1:10 with distilled water to make 1X Assay Buffer.
2. Make serial dilutions of Recombinant LMW-PTP/ACP1 with 1X Assay Buffer (ex. 100%, 50%, 25%, 12.5%, 6.25%, 3.13% 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 (ng/assay)
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.

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:
   \[
   \text{Activity (reaction velocity)} = \frac{\text{Fluorescence Intensity of Test Sample}}{\text{Reaction time (min)}}
   \]
   *NOTE*: Usually, the linear range is from 0 to 30 min. 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.