SensoLyte® MFP Acid Phosphatase Assay Kit *Fluorimetric*

Revision Number: 1.1  Last updated: October 2014

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>AS-71231</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kit Size</td>
<td>500 Assays (96-well) or 1250 Assays (384-well)</td>
</tr>
</tbody>
</table>

- **Convenient Format:** Complete kit includes all the assay components.
- **Optimized Performance:** Optimal conditions for detecting acid phosphatase activity.
- **Enhanced Value:** Less expensive than the sum of individual components.
- **High Speed:** Minimal hands-on time.
- **Assured Reliability:** Detailed protocol and references are provided.

### Kit Components, Storage and Handling

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component A</td>
<td>MFP Ex/Em= 470 nm/510 nm</td>
<td>250 µL, 1 vial</td>
</tr>
<tr>
<td>Component B</td>
<td>Assay buffer</td>
<td>30 ml</td>
</tr>
</tbody>
</table>

**Other Materials Required (but not provided)**

- 96 or 384-well microplate: Black, flat-bottom 96-well or 384-well plate with non-binding surface.
- **Fluorescence microplate reader:** Capable of detecting emission at 510±20 nm with excitation at 470±20 nm.

**Storage and Handling**

- Store all components at -20°C.
- Component B can be stored at room temperature for convenience.
Introduction

The change in acid phosphatase level and activity is involved in a variety of physiological and pathological events, such as prostate puberty, rheumatoid arthritis\(^1\), bone-resorption related diseases\(^2\), and diabetes\(^3\). Acid phosphatase is also a serum marker of tumor bone metastasis\(^4,5\).

The SensoLyte® MFP Acid Phosphatase Assay Kit is optimized to measure acid phosphatase activities using MFP as a fluorogenic substrate. Upon dephosphorylation by phosphatases, MFP generates MF, which has bright green fluorescence even in acidic buffer. The signal can be monitored continuously at excitation/emission=470 nm/510 nm.

Protocol

Note: Warm all kit components to room temperature before starting the experiment.

1. Prepare MFP reaction solution freshly for each experiment.
   1.1 Dilute MFP (Component A) 1:100 in assay buffer (Component B).
   
   Table 1. MFP reaction solution for one 96-well plate (100 assays)
   
<table>
<thead>
<tr>
<th>Components</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFP (100X, Component A)</td>
<td>50 µL</td>
</tr>
<tr>
<td>Assay Buffer (Component B)</td>
<td>4.95 mL</td>
</tr>
<tr>
<td>Total volume</td>
<td>5 mL</td>
</tr>
</tbody>
</table>

2. Detect the activity of acid phosphatase.
   2.1 Add 50 µL/well (black 96-well plate) or 20 µL/well (black 384-well plate) of acid phosphatase-containing sample. Include a non-phosphatase-containing sample as a negative control.
   2.2 Add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of the MFP reaction solution. Mix the reagents by gently shaking the plate for 30 sec.
   2.3 Measure fluorescence signal:
      
      For kinetic reading: Immediately start measuring fluorescence intensity at Ex/Em=470 ±20 nm/510 ±20 nm continuously and record data every 5 min for 30 to 60 min.
      
      For end-point reading: Incubate the reaction at the desired temperature for 30 to 60 min, and keep away from light. Measure fluorescence intensity at Ex/Em=470 ±20 nm/510 ±20 nm.

References