



## SensoLyte<sup>®</sup> MFP Acid Phosphatase Assay Kit \*Fluorimetric\*

Revision Number: 1.1	Last updated: October 2014
<b>Catalog #</b>	<b>AS-71231</b>
<b>Kit Size</b>	500 Assays (96-well) or 1250 Assays (384-well)

- **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

Component	Description	Quantity
Component A	MFP Ex/Em= 470 nm/510 nm	250 $\mu$ L, 1 vial
Component B	Assay buffer	30 ml

### 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 $\pm$ 20 nm with excitation at 470 $\pm$ 20 nm.

### Storage and Handling

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

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## 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<sup>1</sup>, bone-resorption related diseases<sup>2</sup>, and diabetes<sup>3</sup>. Acid phosphatase is also a serum marker of tumor bone metastasis<sup>4,5</sup>.

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.

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## 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)**

Components	Volume
MFP (100X, Component A)	50 µL
Assay Buffer (Component B)	4.95 mL
Total volume	5 mL

### 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.

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## References

1. R. Hayman and T. M. Cox, *Cell Biochem.Funct.* **22**, 275-280 (2004).
2. J. Janckila et al., *J. Leukoc. Biol.* **77**, 209 (2005).
3. N. Bottini et al., *Metabolism* **53**, 995-1001 (2004).
4. T. Y. Chao et al., *J. Biomed. Sci.* **11**, 511-516 (2004).
5. K. Seki et al., *Am. J. Surg. Pathol.* **28**, 1384-1388 (2004).