SensoLyte® MFP Acid Phosphatase Assay Kit *Fluorimetric*

Revision# 1.2	*Last Updated: July 2021*
Catalog #	**AS-71231**
Kit Size	500 Assays (96-well plate)

- ***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 µL, 1 vial
Component B	Assay buffer	30 ml

Other Materials Required (but not provided)

- 96-well microplate: Black, flat-bottom 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)

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 of acid phosphatase-containing sample. Include a sample that doesn't contain phosphatase as a negative control.

2.2 Add 50 µL/well 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

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).