SensoLyte® FDP Protein Phosphatase Assay Kit *Fluorimetric*

Revision Number:1.1	Last updated:October-2014
Catalog #	**AS-71100**
Kit Size	500 Assays (96-well) or 1250 Assays (384-well)

- ***Convenient Format:*** Complete kit includes all the assay components.
- ***Optimized Performance:*** Optimal conditions for detecting protein 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	FDP, Ex/Em= 485±20/528±20 nm upon phosphate group removal	1 vial
Component B	Assay buffer, pH 6.5	60 ml
Component C	10X Lysis buffer	50 ml
Component D	Triton X-100	500 µL
Component E	Stop solution	30 ml
Component F	1 M DTT	100 µl
Component G	DMSO	500 µl

Other Materials Required (but not provided)

- 96-well or 384-well microplate: Black, flat-bottom microplates with non-binding surface.
- Fluorescence microplate reader: Capable of detecting emission at 528±20 nm with excitation at 485±20 nm.
- Protease Inhibitors: Aprotinin, Leupeptin, PMSF and Pepstatin A

Storage and Handling

- Store all kit components at -20°C.
- Components B, C, D, E and G can be stored at room temperature for convenience.

Introduction

Protein phosphorylation / dephosphorylation, a potent and versatile mechanism for the regulation of protein activity, plays a key role in signal transduction and cellular function modulations. Consequently, protein phosphatases have received attention as potential drug-screening targets.

The SensoLyte® FDP Protein Phosphatase Assay Kit provides a convenient fluorimetric assay for measuring the activity of protein phosphatases, such as protein tyrosine phosphatases, serine/threonine phosphatases, Na^+/K^+ ATPase[1], and plasma membrane Ca^{2+}-ATPase. Fluorescein, the final hydrolytic product of FDP, has a high extinction coefficient and emission quantum yield, therefore providing better assay sensitivity than the colorimetric counterparts such as *p*NPP. The signal of fluorescein can be easily read by a fluorescence plate reader at Ex/Em=485±20/528±20 nm.

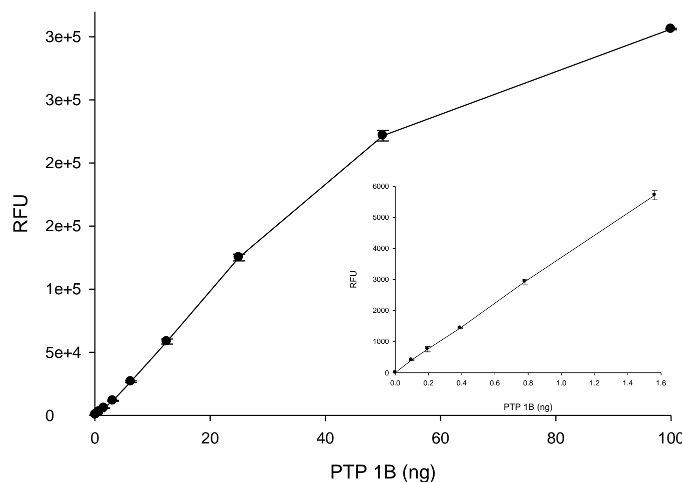

Figure 1. The assay sensitivity for protein tyrosine phosphatase 1B (PTP 1B)
Recombinant PTP 1B was serially diluted in assay buffer, pH 6.5, and its activity was measured according to the protocol. The assay can detect as low as 0.1 ng of PTP 1B.

Protocol

Note 1: Warm all kit components to room temperature before starting the experiments.
Note 2: FDP is a generic phosphatase substrate. In order to measure the activity of a specific phosphatase in biological samples, the enzyme should be purified by immuno-affinity purification or other methods.

1. Prepare protein phosphatase-containing sample.

1.1 For protein phosphatase-containing biological samples, please refer to **Appendix I** for the preparation of cell extract or tissue extract.

1.2 For purified protein phosphatase, dilute the purified enzyme in assay buffer (Component B) to the appropriate concentration.

Note: The activity of protein phosphatase can be preserved better if the purified enzyme is diluted with assay buffer to which bovine serum albumin is added to a final concentration of 1 mg/mL. Keep the enzyme on ice before the experiment. Avoid vigorous agitation of the enzyme.

2. Prepare stock solution (for first time preparation only).

2.1 FDP stock solution: Reconstitute FDP (Component A) by adding 250 µL of DMSO (Component G) into the vial. Mix the reagents well. Stock solution is good for 3-4 months if stored at -20°C.

3. Prepare FDP reaction solution fresh according to Table 1.

Table 1. FDP reaction solution for one 96-well plate (100 assays)

Components	Volume
FDP stock solution (Component A)	50 µL
Assay Buffer (Component B)	4.935 mL
1 M DTT (Component F)	15 µL
Total volume	5 mL

Note: The assay buffer (Component B) is optimized for protein tyrosine phosphatase. Some protein phosphatases require a unique assay buffer. Please check the Appendix II for some references.

4. Start the protein phosphatase detection.

4.1 Add 50 µL/well (black 96-well plate) or 20 µL/well (black 384-well plate) of the protein phosphatase-containing sample. Include a non-phosphatase-containing sample as a negative control.

4.2 Add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of the FDP reaction solution. Mix the reagents by gently shaking the plate for 30 sec.

4.3 Measure fluorescence signal:

- For kinetic reading: Immediately start measuring fluorescence intensity at Ex/Em=485±20nm/528±20nm and continuously 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 protect from light. Optional: Add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of stop solution (Component E). Measure fluorescence intensity at Ex/Em=490±20/520±20 nm.

Note: The signal of fluorescein is pH-sensitive with maximum intensity at pH ≥6.0. If the assay is performed at pH < 6.0, the pH-adjusting stop solution must be added before measuring the fluorescence.

Appendix I

Prepare cell extract for protein phosphatase assay.

- Prepare 1X lysis buffer by adding 1 mL of 10X lysis buffer (Component C) to 9 mL of deionized- water.
- Gently wash cells with 1X lysis buffer twice.
- Add 20 µL of Triton X-100 (Component D) to 10 mL of 1X lysis buffer. Add protease inhibitors to a final concentration of 10 µg/ml Aprotinin, 10 µg/mL Leupeptin, 100 µM PMSF and 10 µg/ml Pepstatin A.
  Note: Protease inhibitors are not provided.
- Add an appropriate amount of the 1X lysis buffer to the cells or cell pellet. Scrape off the adherent cells or resuspend the cell pellet. Collect the cell suspension in a microcentrifuge tube.
- Incubate the cell suspension at 4°C for 10 min with agitation.
- Centrifuge the cell suspension at 2,500x g for 10 min at 4°C.
- Collect the supernatant to perform the protein phosphatase assay.

Prepare tissue extract for protein phosphatase assay.

- Prepare 1X lysis buffer by adding 1 mL of 10X lysis buffer (Component C) and 20 µL of Triton X-100 (Component D) to 9 mL of deionized water. Add protease inhibitors to a final concentration of 10 µg/ml Aprotinin, 10 µg/mL Leupeptin, 100 µM PMSF, and 10 µg/ml Pepstatin A.
  Note: Protease inhibitors are not provided.
- Add an appropriate amount of 1X lysis buffer to the tissue sample and homogenize.
- Centrifuge the tissue sample at 10,000x g for 10 min at 4°C.
- Collect the supernatant for protein phosphatase assay.

Appendix II. References for protein phosphatase assay buffer

Phosphatases	Assay Buffer
CD45, PTP1B	50 mM Bis-tris, pH 6.5, 2 mM EDTA, 5 mM DTT, 0.05% Brij35[2]
PP1	100 mM Tris-HCl, pH 7.5, 4 mM DTT, 0.2 mM EDTA, 0.5 mM $MnCl_2$, 0.4 mg/mL BSA[3]
PP2A	40 mM Tris-HCl, pH 8.4, 34 mM $MgCl_2$, 4 mM EDTA, 4 mM DTT[4]
Na+/K+ ATPase	80 mM Tris-HCl, pH 7.2, 4 mM $MgCl_2$, 0.5 mM EGTA, 5 mM creatinine phosphate, activated by 10 mM KCl
PTEN	100 mM Tris-HCl, pH 8, 10 mM DTT[5]
PP2Cα	50 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 0.5 mM DTT, 60 mM $MgCl_2$[6]

References

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