SensoLyte® *p*NPP Protein Phosphatase Assay Kit **Colorimetric**

Revision Number:1.1	*Last Updated: October 2014*
Catalog #	**AS-71105**
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	*p*NPP, absorbance=405 nm upon phosphate group removal	1 vial
Component B	Assay buffer	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

Other Materials Required (but not provided)

- 96-well or 384-well microplate: Clear, flat-bottom microplates with non-binding surface.
- Absorbance microplate reader: Capable of detecting absorbance at 405 nm.
- Protease Inhibitors: Aprotinin, Leupeptin, PMSF and Pepstatin A.

Storage and Handling

- Store kit components at -20°C
- Components B, C, D, and E 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 great attention as potential drug-screening targets.

*p*NPP (*p*-Nitrophenyl phosphate) is a colorimetric substrate for measuring the activity of protein phosphatases, such as protein tyrosine phosphatases, serine/threonine phosphatases, Na^+/K^+ ATPase, and plasma membrane Ca^{2+}-ATPase. Upon dephosphorylation by phosphatases, *p*NPP turns yellow and can be detected at absorbance=405 nm.

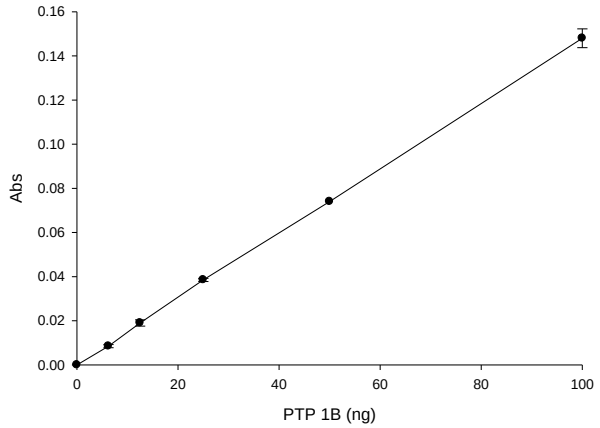

Figure 1. The assay sensitivity in measuring protein tyrosine phosphatase 1B (PTP 1B).
Recombinant PTP 1B was serially diluted in assay buffer, pH 6.5, and its activity was measured according to the protocol. The assay can detect as low as 10 ng of PTP 1B.

Protocol

Note 1: Warm all kit components to room temperature before starting the experiment.
Note 2: Since *p*NPP is a generic phosphatase substrate, in order to measure the activity of the phosphatase of interest, your sample has to be purified by immunoaffinity or other methods before measuring its activity using *p*NPP.

1. Prepare protein phosphatase containing sample.

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

1.2 For purified protein phosphatase, dilute the 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 containing 1 mg/mL of bovine serum albumin. Keep enzyme on ice before the experiment. Avoid vigorously mixing of the enzyme.

2. Prepare *p*NPP reaction mixture.

3.1 First time preparation only: Reconstitute by adding 250 µL of deionized water into the *p*NPP vial (Component A). Completely dissolve *p*NPP. The stock solution will be good for 3-4 weeks if stored at -20°C.

3.1 *p*NPP reaction mixture: Prepare freshly according to Table 1.

Table 1. *p*NPP reaction solution for one 96-well plate (100 assays)

Components	Volume
*p*NPP stock solution (100X, 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) works for protein tyrosine phosphatase. Since some protein phosphatases require their unique assay buffer, you may use your own formulated buffer. Please refer to Appendix II for some references.

3. Start the protein phosphatase detection.

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

3.2 Add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of *p*NPP reaction mixture. Mix the reagents by gently shaking the plate for 30 sec.

3.3 Measure absorbance signal:

- For kinetic reading: Immediately start measuring absorbance reading at 405 nm continuously and record data every 5 min for 30 min.
- For end-point reading: Incubate the reaction at the desired temperature for 30 to 60 min. Optional: Add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of stop solution (Component E). Shake the plate on a plate shaker for 1 min before the reading. Measure absorbance at 405 nm.

Appendix I

Prepare cell extract for protein phosphatase

- Prepare 1X lysis buffer by adding 1 mL of 10X lysis buffer (Component C) to 9 mL of deionized- water.
- Wash cells with 1X lysis buffer twice gently.
- 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 above lysis buffer to cells. 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 under agitation.
- Centrifuge the cell suspension at 2500 X g for 10 min at 4°C.
- Collect the supernatant for the protein phosphatase assay.

Prepare tissue extract for protein phosphatase.

- 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 10,000 X 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[1]
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[2]
PP2A	40 mM Tris-HCl, pH 8.4, 34 mM $MgCl_2$, 4 mM EDTA, 4 mM DTT[3]
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[4]
PP2Cα	50 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 0.5 mM DTT, 60 mM $MgCl_2$[5]

References

1. Huang, Z. et al. *J. Biomol. Screen.* **4**, 327 (1999).
2. Heresztyn, T. et al. *Environ. Toxicol.* **16**, 242 (2001).
3. Takai, A. et al. *Biochem. J.* **287** (Pt 1), 101 (1992).
4. Maehama, T. et al. *Anal. Biochem.* **279**, 248 (2000).
5. Marley, A.E. et al. *Biochem. J.* **320** (Pt 3), 801 (1996).