QuantiChrom™ Pyrophosphatase Assay Kit (DPPT-100)

Quantitative Colorimetric Determination of Pyrophosphatase Activity at 620nm

DESCRIPTION

INORGANIC PYROPHOSPHATASE(E.C.3.6.1.1) catalyzes the hydrolysis of phosphoester bonds on inorganic pyrophosphate [$P_2O_7^{4-}$], thereby releasing two orthophosphate molecules. Family I PPases are essential enzymes found in all kingdoms of life and are responsible for maintaining the correct pyrophosphate equilibrium necessary to carry out nucleic acid and protein synthesis, and facilitate fatty acid β-oxidation. Simple, direct and automation-ready procedures for measuring pyrophosphatase activity are very desirable. BioAssay Systems' DPPT-100 assay is based on our proprietary phosphate assay kit (POMG-25H). The color intensity, measured at 620 nm, is proportionate to the amount of phosphate released from pyrophosphate hydrolysis.

KEY FEATURES

Safe and sensitive. Non-radioactive assay. Use as little as 10 µL samples. Linear detection range in 96-well plate: 1.0 to 20 U/L activity.

Fast and convenient. The procedure involves addition of a single working reagent and incubation for 60 min. Room temperature assay. No 37°C incubator is needed.

High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For detection and quantification of pyrophosphatase enzyme activity.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer (pH 7.2):	8 mL	**POMG Reagent A:**	2.5 mL
Pyrophosphate:	100 µL	**POMG Reagent B:**	120 µL
Standard:	120 µL 1 mM Phosphate		

Storage Conditions. The kit is shipped at room temperature. Store all components at 4°C upon receiving. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Samples. Phosphate-containing detergents and buffers must be avoided in the preparation of pyrophosphatase samples as the assay is extremely sensitive to free phosphate. If the OD620nm for enzyme alone with the color reagent is higher than 0.2, we recommend removing free phosphate by at least 3 washes with a 10 kDa NMWL membrane filter (e.g. Amicon® Ultra 0.5 mL Ultracel or similar). Avoid using samples with EDTA and metals such as Zn, Co, Mn, and Ca. High protein concentrations can interfere with the Color Development through precipitation. For an enzyme sample of unknown activity, it is prudent to run a serial dilution in dH_2O to bring the activity within the detection range and choose dilutions at which no precipitation occurs.

1. ***Enzyme Reaction***. Pipette 10 µL of pyrophosphatase sample into separate wells of a clear flat-bottom 96-well plate. Reserve one well for each sample as a Blank (10 µL of buffer, no enzyme).

   Prepare enough Working Reagent (*WR)* by mixing 1 µL of the pyrophosphate with 80 µL of the Assay Buffer per assay well.

   Initiate the reaction by adding 70 µL of the *WR* into each well. Tap plate briefly to mix the reaction mixture.

   Incubate the plate at room temperature or the desired temperature for 30 minutes.

2. ***Phosphate Determination***: Prepare a 40 µM phosphate premix solution by pipetting 20 µL of the 1 mM phosphate standard to 480 µL of dH_2O. Number the tubes and dilute standards as shown in the table.

   Pipette 80 µL of each standard in duplicate into separate wells of the assay plate.

No	Premix + H_2O	Final Vol (µL)	[Phosphate] (µM)
1	200µL + 0µL	200	40
2	120µL + 80µL	200	24
3	60µL + 140µL	200	12
4	0µL + 200µL	200	0

   Prepare Color Development Reagent. Each well requires 20 µL Color Development Reagent, prepared by mixing 100 vol of POMG Reagent A with 1 vol of POMG Reagent B, e.g. 1 mL of Reagent A and 10 µL of Reagent B. Prepared reagent is stable for at least 1 day at room temperature.

   At 30 minutes in Step 1, add 20 µL of the Color Development Reagent to each well. Mix gently by tapping the plate and incubate for an additional 30 min at room temperature for color development.

3. Measure absorbance at 600 nm - 660nm (peak 620 nm) with a plate reader.

   Note: If the observed phosphate concentration is equal to or greater than the 40 µM Phosphate Standard, dilute the enzyme extract in the buffer and repeat the assay.

Calculation: The sample pyrophosphatase activity is calculated:

$$\text{Enzyme Activity} = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope}} \times \frac{\text{Reaction Vol (L)}}{t\ \text{(min)} \times \text{Sample Vol (L)}} \times n\ \text{(U/L)}$$

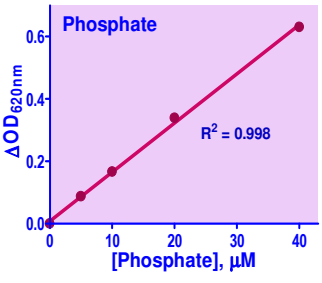
$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope}} \times 0.267 \times n\ \text{(U/L)}$$

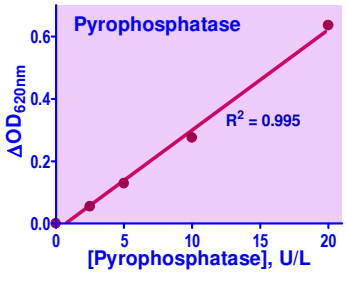
Where OD_{SAMPLE} and OD_{BLANK} are the sample and blank absorbances, respectively, and the Slope is the Slope (μM^{-1}) of the phosphate standard curve. Reaction vol and Sample vol are 80 µL and 10 µL, respectively. t is the reaction time (30 min). n is the sample dilution factor.

Unit definition: one unit (U) of enzyme catalyzes the production of 1 µmole of orthophosphate per minute under the assay conditions (pH 7.2).

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting (multi-channel) devices. Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader. 10kDa NMWL membrane filters.





Left: Phosphate standard curve. *Right*: a titration of pyrophosphatase using the standard 96well assay procedure. The enzyme used was pyrophosphatase from baker's yeast (Sigma cat# I1643-100 UN).

LITERATURE

1. Yang, L., Liao, R. Z., Yu, J. G., & Liu, R. Z. (2009). DFT study on the mechanism of Escherichia coli inorganic pyrophosphatase. *The Journal of Physical Chemistry B*, *113*(18), 6505-6510.

2. Carman, G. M., & Han, G. S. (2006). Roles of phosphatidate phosphatase enzymes in lipid metabolism. *Trends in biochemical sciences*, *31*(12), 694-699.

3. Nakano, T., et al (1999) Purification and Characterization of Phytase from Bran of *Triticum aestivum* L. cv. Nourin #61. *Food Sci. Technol. Res.* 5(1): 18.

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