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User's Manual and Instructions

PBlue Phosphate Assay Kits (Z5030013)

DESCRIPTION

The PBlue Phosphate Assay Kit is based on a proprietary formulation of the malachite green dye. The PBlue reagent forms a blue colored complex with free orthophosphate. The rapid color formation from the reaction can be conveniently measured on a spectrophotometer (600 - 660 nm) or on a plate reader. The non-radioactive colorimetric assay kits have been optimized to offer superior sensitivity and prolonged shelf life. The assay is simple and fast, involving a single addition step for phosphate determination. Assays can be performed in tubes, cuvettes or multi-well plates. The assays can be conveniently executed in 96-well plates for high-throughput screening of enzyme inhibitors.

KEY FEATURES

Reagent very stable. Due to our innovative formulation, no precipitation of reagent occurs. Therefore no filtration of reagent is needed prior to assays, as is often required with other commercial kits.
High sensitivity and wide detection range: detection of as little of 20 pmoles of phosphate and useful range between 0.4 μM and 50 μM phosphate.
Fast and convenient: single reagent "mix-and-measure" assay allows quantitation of free phosphate within 30 minutes.
Compatible with routine laboratory and HTS formats: assays can be performed in tubes, cuvettes or microplates, on spectrophotometers and plate readers.
Robust and amenable to HTS: Z' factors of 0.7 to 0.9 are observed in 96-well plates. Can be readily automated on HTS liquid handling systems.

APPLICATIONS

Phosphatase Assays: liberation of phosphate from peptide, protein or small molecule substrate.
Lipase Assays: liberation of phosphate from phospholipids
Nucleoside Triphosphatase Assays: liberation of phosphate from nucleoside triphosphates (ATP, GTP, TTP, CTP etc).
Quantitation of Phosphate in phospholipids, proteins and DNAs, etc.
Drug Discovery: high-throughput screen for phosphatase inhibitors.

KIT CONTENTS

Catalog #	Size (assays)	Reagent	Standard
Z5030013	500	50 mL	1 mL 1mM phosphate

Storage conditions. The PBlue Reagent and standard are stable for 12 months when stored at 4°C.

This protocol can be downloaded online at www.biochain.com.

Precautions: reagents are for research use only. This reagent contains 0.44 M sulfuric acid. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Procedure using 96-well plate:

***Important**: The reagent must be brought to room temperature and well shaken before use. The PBlue reagent is highly sensitive to phosphate. It is important that all enzyme preparations and assay buffers not contain free phosphate. Lab detergents may contain high levels of phosphate. Make sure that lab wares are washed thoroughly with distilled water and free from contaminating phosphate.*

1. Dilution of phosphate standards. Prepare a 1000 μL 40 μM phosphate Premix solution by mixing 40 μL 1 mM phosphate standard with 960 μL distilled water. Number the tubes. Prepare concentration standards by diluting the Premix as shown in the The phosphate concentrations in the tubes are given below.

Transfer 50 μL diluted standard in duplicate to wells in a clear-bottom 96-well plate. Store diluted standard at 4°C for future use.

No	Premix + H_2O	Final Vol (μL)	Phosphate Conc (μM)	pmoles Phosphate in 50 μL
1	200μL + 0μL	200	40	2,000
2	160μL + 40μL	200	32	1,600
3	120μL + 80μL	200	24	1,200
4	80μL + 120μL	200	16	800
5	60μL + 140μL	200	12	600
6	40μL + 160μL	200	8	400
7	20μL + 180μL	200	4	200
8	0μL + 200μL	200	0	0

2. Transfer 50 μL test sample (e.g. enzyme reaction) in duplicate into wells of the microplate. In the case of enzyme reactions, the reaction may be terminated by either adding a specific inhibitor, or can be stopped directly by the addition of the PBlue Reagent. Reaction buffer can be added as a blank control for the samples.

3. Add 100 μL of the PBlue Reagent to each well. Mix by tapping the plate.

4. Incubate for 30 min at room temperature for color development.

5. Measure absorbance at 620 nm (600 nm - 660nm) on a plate reader.

Procedure using Cuvette:

For cuvette assays, add 800 μL Reagent to 400 μL sample and standards. Perform the assay as described for the microplate assay.

GENERAL CONSIDERATIONS

Incubation time. The chromogenic reaction is completed within 30 min. The signal is best read between 30 and 60 min.
Precipitation may occur at high concentrations of phosphate (>100 μM), or in the presence of high concentrations of e.g. proteins and metals. If precipitation occurs, dilute samples in distilled water and repeat the assay.
Enzyme reaction buffer. Because any exogenous free phosphate would interfere with the assay, it is important to ensure that the protein preparation, the reaction buffer and lab wares employed in the assay should not contain free phosphate. This can be conveniently checked by adding the Reagent to the buffer and measuring the color formation.

DATA ANALYSIS

Plot pmoles phosphate versus OD_{620nm} for the standard curve. Use linear regression analysis to determine amount of free phosphate in the test samples.

LITERATURE

High-throughput Screening

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PBlue Phosphate Assay Kits

Assays for phosphatases, lipases/phospholipids, nucleoside triphosphatases and phosphate in proteins and DNAs.

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TECHNICAL NOTES

The PBlue Phosphate Assay Kits have been optimized and formulated to provide a sensitive, convenient and robust determination of free phosphate liberated from enzyme reactions and natural sources. Key features of the kits are as follows:

Reagent very stable. Due to our innovative formulation, no precipitation of reagent occurs. Therefore no filtration of reagent is needed prior to assays, as is often required with other commercial kits.
Safe. Non-radioactive assay.
High sensitivity and wide detection range: detection of as little of 20 pmoles of phosphate and useful linear range is between 0.4 μM and 50 μM phosphate.
Fast and convenient: single reagent "mix-and-measure" assay allows quantitation of free phosphate within 30 minutes.
Compatible with routine laboratory and HTS formats: assays can be performed in tubes or microplates, on spectrophotometers and plate readers.
Robust and amenable to HTS: Z' factors of 0.7 to 0.9 are observed in 96-well plates. Can be readily automated on HTS liquid handling systems.

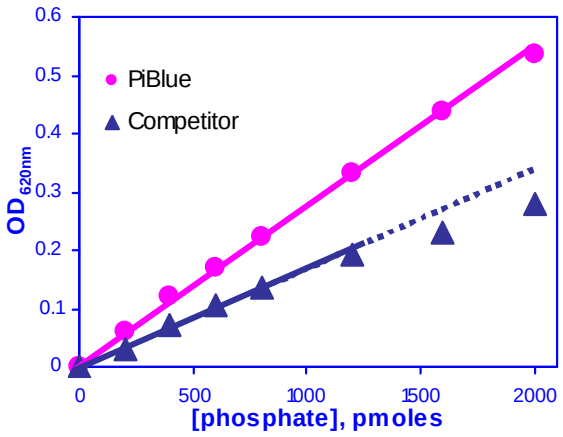

Figure 1. Phosphate standard curve in 96-well plate assay. 100 μL PBlue Reagent was added to 50 μL phosphate standard. After 30 minutes incubation, the OD at 620 nm was measured on a plate reader. Useful detection range was 0.4 μM to 50 μM phosphate. The detection limit estimated from blank control values was 20 pmoles. Coefficient of variance was generally below 6%. Z' factor was > 0.7. The same assay was performed using a competitor's product.