ABS_Bio™ Phosphate Detection Kit

(Cat# K198-500; 500 Colorimetric assays; store kit at 4℃)

Introduction

Phosphate is an essential component in living organisms and contributes to a variety of biological functions. Phosphate is also important in the transport of cellular energy, nucleic acid metabolism, and signal transduction.

The ABS_Bio™ Phosphate Detection Kit provides a fast, reproducible, colorimetric method for measuring inorganic free phosphate in aqueous solutions. Formulated single reagent allows quantitation of free phosphate within 30 minutes. Applications for this assay include quantification of phosphorylation and phosphate release from protein phosphatase substrates. This assay is a reliable and suitable detecting and quantifying minimal amounts of inorganic free phosphate and is amenable to high-throughput screening applications. The assay method is based on the formation of a complex between malachite green, molybdate and free orthophosphate, and the colored product is determined around 630 nm (600-650nm) directly proportional to the phosphate in the biological and environment samples. The kit has been used for assaying phosphatase, phospholipase or lipase, nucleoside triphosphatase (ATPase, GTPase, etc.). The kit is supplied with enough reagents for 500 tests in 96-well plate assay, linear detection range of 0.1-100 µM phosphate. It could easily be modified for use in 384-well or cuvette-based assays.

Kit Components (500 tests)

Reagent : 50 mL Standard (1 mM Phosphate): 1 mL

Storage and Handling: Store kit at 4℃. Shelf Life: 12 months after receipt. Warm Reagents to room temperature before use. The reagent is strong acid solution and should be handled like any laboratory acid.

Protocol

1. Sample preparation

It is important to ensure that the protein preparation, the reaction buffer and lab wares employed in the assay should not contain free phosphate. It is highly recommended to use disposable plastic lab ware for all samples, standards and reagents to avoid contamination. No pretreatment is necessary for biological and environment samples, directly add 1-50 µL of sample to the duplicate wells, and bring samples to final volume of 50 µL with dH_2O for the assay.

2. Standard Preparation

Prepare 50 µM phosphate STD by add 50 µL 1 mM STD into 950 µL dH_2O, then following the table to generate 50, 30, 15 and 0 µM phosphate standards.

phosphate std(µL)	dH_2O(µL)	final phosphate (µM)
200	0	50
120	80	30
60	140	15
0	200	0

For cuvette format Assay	
Sample/std volume(µL)	Reagent volume(µL)
250	500
For 384-well format Assay	
Sample/std volume(µL)	Reagent volume(µL)
20	40

Add 50 µL of appropriate standards into duplicate wells in the 96-well clear flat-bottom plate. The blank control containing water or reaction buffer only.

3. Reaction

Add 100 µL reagent into each well containing the standard and samples, and tap plate lightly to mix well.

4. Measurement

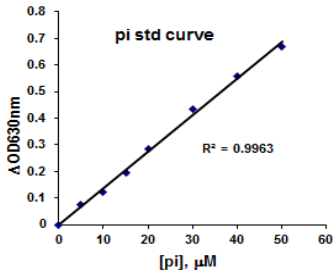
Incubate for 20-30 min. at 20-37℃, prevent light. Read the optical density at 630 nm (OD600-650nm).

5. Calculation

Average the duplicate OD630 nm for standard and sample. Create a standard curve by subtracting the mean OD's for the blank. Plot the phosphate standard Curve. Determine the slope and calculate the phosphate concentration of samples.

[Phosphate]= (OD_{sample}-OD_{blank})/Slope (µM)

Typical Standard Curve



Phosphate standard in 96 wells-plate assay.

Validation Data	
Human Serum	522-564 µM
Human Urine	340-379 µM
Tap water	0.3-9.6 µM
Fat free milk	586-673 µM
Detection Limit	0.11 µM

Always run your own standard curves for calculation of results.

Sensitivity and Limit of Detection

Sensitivity was determined as 0.030 μM. The Limit of Detection was determined as 0.11, and linear detection range up to 100 μM in 96-well plate assay. Samples with values above 100 μM should be dilute with dH_2O, re-assayed, and multiply results by dilution factor. 1 mg/dL phosphate equals 105.3 μM.

Interferences

If sample protein concentration is more than 1 mg/mL or ATP/GTP is more than 0.25 mM, it may accelerate precipitation of dye complexes, needs to dilute the sample with dH_2O prior to the assay.

References

Itaya, K. et al. 1966, Clin Chem 14:361-366
Baykov, AA. et al. 1988, Analytical Biochemistry. 171:266-270.
Carter, SG. et al. 1982, J. Biochem Biophys Methods. 7:7-13.

Related Products:

ALP Colorimetric Detection Kit (#K109-200)
ALP Fluorimetric Detection Kit (#K110-200)
ALP Substrate Solution (#C8040)
BCIP/NBT Substrate system (#C8041)
Creatinine Detection Kit (#K148-100)
Glucose Detection Kit (#K188-100)
Glutathione Detection Kit (#K140-100)
Glutathione Peroxidase Detection Kit (#K143-100)
Glutathione Reductase Detection Kit (#K146-100)
HRP Fluorimetric Detection Kit (#K210-100)
HRP Substrate system (#C8042)
Hydrogen Peroxide Detection Kit (#K125-100)
Lactate Detection Kit (#K207-100)
NAD/NADH Detection Kit (#K120-100)
NADP/NADPH Detection Kit (#K123-100)
Nitric Oxide Detection Kit (#K116-100)
Phosphatase Detection Kit (#K196-500)
Urea Detection Kit (#K158-200)
pNPP substrate solution (#C8071)
TBARS Detection Kit (#K145-100)
TMB substrate solution(#C8010)