Phosphate Colorimetric Assay Kit

(Catalog #BN00663; 500 assays; Store Kit at Room Temp.)

I. Introduction:

Phosphate is one of the most important of the inorganic ions in biological systems. It functions in a variety of roles. One of the most important roles is as a molecular switch, turning enzyme activity on and off through the mediation of the various protein kinases and phosphatases in biological systems. Phosphate is also of great importance in mineralization processes and is a primary stimulus of algal blooms frequently found in bodies of fresh water, due to run-off from areas of high fertilizer use. The newly designed Phosphate Colorimetric Assay Kit provides an easy, quick and sensitive means of assessing phosphate over a wide range of concentrations. The assay utilizes a proprietary formulation of malachite green and ammonium molybdate which forms a chromogenic complex with phosphate ion giving an intense absorption band around 650 nm. Phosphate concentrations between 1 µM and 1 mM, with a lower limit of detection of approximately 0.1 nmol, can be directly determined. The Phosphate Colorimetric Assay Kit provides 500 assays using microtiter plates or 100 assays using 1 ml cuvettes.

II. Kit Contents:

Components	BN00663	Cap Code	Part Number
Phosphate Reagent	15 ml	WM	BN00663-1
Phosphate Standard (10 mM)	0.5 ml	Yellow	BN00663-2

III. Reconstitution of Reagents:

Phosphate Reagent: Ready to use as supplied and may be kept at room temperature. There may be a small amount of precipitate visible which doesn't affect the assay.

IV. Assay Protocol:

1. **Phosphate Standard Curve:** Dilute 10 µl of the 10 mM Phosphate Standard to 990 µl dH_2O, mix well to generate 100 µM working Phosphate Standard. Add 0, 10, 20, 30, 40, 50 µl of the 100 µM working Phosphate Standard to individual wells. Adjust the volume to 200 µl with dH_2O to generate 0, 1, 2, 3, 4, 5 nmol of Phosphate standard.
2. **Preparation of sample:** No sample pretreatment is necessary. Add between 0-200 µl of sample for the assays and bring the well volume to 200 µl with distilled water. If the approximate phosphate concentration is not known, we recommend widely different sample volumes (1, 10, 100 µl) be tested.

   The absorbance of samples should be in the linear range of the standard curve (0-5 nmol/well). If they fall outside of this range, samples should be diluted and rerun or smaller sample volumes be used. The detection limit of the assay is approximately 0.1 nmol per well (1 µM) of Phosphate.

3. **Reaction:**
   1) Add 30 µl Phosphate Reagent to all standard and sample wells, mix well.
   2) Cover the plate and incubate at room temperature for 30 min.
   3) Read the absorbance at 650 nm using a plate reader. The color is stable for several hrs.
4. **Protocol for using 1.0 ml cuvettes:** Increase all reaction components 5X when using 1 ml cuvettes. The 1 ml total reaction mixture will contain 0-25 nmol phosphate (0-500 µl), 150 µl of Phosphate Reagent and made up to 1.0 ml with distilled water. Incubate at room temperature for 30 min then read at 650 nm.

5. Calculations:

1) Plot standard curve: Plot absorbance at 650 nm as a function of Phosphate concentration.

2) Determine sample Phosphate concentration:

$$\text{Phosphate concentration} = \frac{\text{(sample absorbance-blank absorbance)}}{\text{(slope of standard curve) x (µl of sample)}}$$

OR

$$\text{Sa/Sv} = \text{nmol/µl or mM Phosphate}$$

Where Sa is the sample amount (in nmoles) read from the standard curve.

Sv is the sample volume (undiluted) added to the wells.

Caution: Many laboratory detergents contain high amounts of phosphates which can adhere to cleaned glassware. It is highly recommended to use disposable plastic labware for all samples, standards and reagents to avoid contamination.

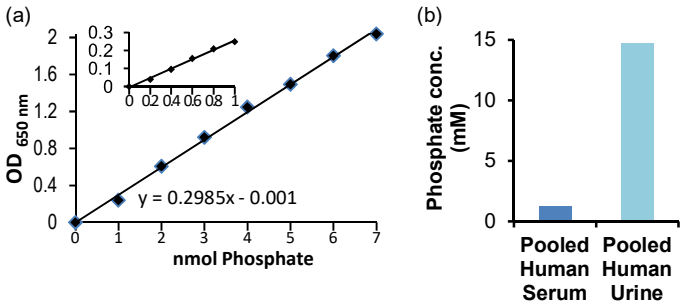

Figure: (a) Phosphate Standard Curve. (b) Quantification of phosphate in pooled human serum (50 µl, 20 times diluted) and pooled human urine (50 µl , 150 times diluted). Assay was performed following the kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold reagent	• Reagent must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		