BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Phosphate Assay Kit (Cat #: A-110)

COMPONENTS: Phosphate Assay Solution: 30 ml, store at 4ºC (for 300 wells)
NaH_2PO_4 Standard (10 mM): 0.5 ml, store at 4ºC

PRODUCT DESCRIPTION: One of the most sensitive colorimetric method for inorganic phosphate assay is based on complex formation of phosphomolybdate with the basic dye Malachite Green (Anal. Biochem. 271:29,1999). The blue green color formed by the reaction can be quantified by measurement of absorbance at 600-660 nm. The kit is stable for several years if stored and handled properly.

PROTOCOL:

Phosphate Assay Solution: Small amounts of greenish aggregates normally appear on the bottom of the Phosphate Assay Solution container during storage. Swirl container to disperse aggregates before pipetting.

Phosphate standard: First dilute the 10 mM NaH_2PO_4 standard 100-fold with dH_2O to obtain a 100 µM phosphate standard, e.g., 1 µl 10 mM NaH_2PO_4 standard + 99 µl dH_2O. Then perform 1:1 serial dilution to obtain 50, 25, and 12.5 µM phosphate standards. Store diluted phosphate standards at 4°C.

Sample preparation

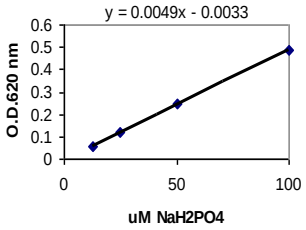
Cell sample: Pellet at least ~10^6 normal saline-washed cells in a microtube. Remove saline completely. Add 0.1 ml ice-cold 10% trichloroacetic acid (TCA) solution (not included) to cell pellet. Disrupt the cell pellet by vigorous trituration and vortexing. Mechanical homogenization can facilitate this step. Agitate extract on ice for 5 min. Clarify sample by centrifugation at ~13,000 rpm for 5 min. Collect supernatant for the assay.

Tissue sample: Tissue sample should be washed with normal saline to remove blood residues. Tissue (~50 mg) is mechanically homogenized in 0.2 ml of ice-cold 10% TCA. Agitate extract on ice for 5 min. Clarify sample by centrifugation at ~13,000 rpm for 5 min. Collect supernatant for the assay.

Serum/plasma sample: Serum/plasma should be deproteinized by TCA precipitation. Mix sample with an equal volume of ice-cold 10% TCA and incubate on ice for 5 min. Clarify sample by centrifugation at ~13,000 rpm for 5 min. Collect supernatant for the assay.

Assay:

1. Use a plain (uncoated) 96-well plate for the assay. First add 20 µl of dH_2O (or an appropriate medium blank) to a well serving as blank well.
2. Add 20 µl of each phosphate standard to a set of wells. Add 20 µl of samples to another set of wells. *Carry out a preliminary analysis to determine the optimal dilution factor for each sample type.*
3. Briefly swirl the Phosphate Assay Solution container before pipetting. Add 100 µl Phosphate Assay Solution to each well followed by a brief gentle agitation. Incubate at room temperature for 10 min. *If turbidity appears in wells, dilute sample with dH_2O and repeat assay.*
4. Measure absorbance (O.D.) at 600 − 660 nm using a plate reader. Subtract blank well reading from each phosphate standard well reading and each sample well reading.
5. Use the subtracted phosphate standard readings to generate a standard plot (see graph below; x= phosphate concentration in µM, y= $O.D._{620\ nm}$).
6. Use the subtracted sample well readings for calculation of sample phosphate concentration as derived from the trend line equation of the standard plot established at the same time.



NOTE:

Phosphate Assay Solution contains hydrochloric acid. Use caution handling the reagent. Please visit the product webpage or contact us for SDS information.