ab65622 Phosphate Assay Kit (Colorimetric)

For quantitative measurement of Phosphate in a variety of biological samples.

For overview, typical data and additional information please visit: www.abcam.com/ab65622
(use abcam.cn/ab65622 for China, or abcam.co.jp/ab65622 for Japan)

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

This product is for research use only and is not intended for diagnostic use.

Materials Supplied and Storage

Store kit at room temperature in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
There may be a small amount of precipitate visible which doesn't affect the assay. You can centrifuge and take the supernatant to avoid taking the precipitate.
Aliquot components in working volumes before storing at the recommended temperature.

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Phosphate Reagent	15 mL	RT	RT
Phosphate Standard/10 mM Phosphate Standard	500 µL	RT	RT

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at OD 650 nm
- TBS
- MilliQ water or other type of double distilled water (ddH2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Tris Buffer or any generic lysis buffer (such as RIPA) – for sample preparation
- mm glass beads/ Dounce homogenizer (if using tissue)
- Sonicator (if using cells)
- (Optional) Protease inhibitors: we recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as a general use cocktail

NOTE: 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 sample, standards and reagents to avoid contamination.

Reagent Preparation

Phosphate Reagent: Ready to use as supplied. Store at room temperature.
NOTE: The phosphate reagent contains malachite green and can form green and black particulates/precipitation in cold temperatures. This is normal and will not affect the assay. If there is significant precipitation, you can centrifuge the phosphate reagent in a table-top micro-centrifuge for 1 minute before use.

Phosphate Standard (10mM): Ready to use as supplied. Store at room temperature.

Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard the working standard dilutions after use as they do not store well.

Prepare a 100 µM Phosphate Standard by diluting 10 µL of the 10mM Phosphate Standard in 990 µL of dH2O.
Using 100 µM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes. Each dilution has enough amount of standard to set up duplicate reading (2 x 200 µL).

Standard #	Volume of Phosphate Standard (µL)	ddH_2O (µL)	Final volume standard in well (µL)	Amount Standard in well (nmol/well)
1	0	600	200	0
2	30	570	200	1
3	60	540	200	2
4	90	510	200	3
5	120	480	200	4
6	150	450	200	5

Sample Preparation

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step as well as the deproteinization step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze samples in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- Add protease inhibitors to sample buffer immediately prior use.

Cell (adherent or suspension) samples:

1. Harvest the number of cells necessary for each assay (initial recommendation = 1 x 10^7 cells).
2. Wash cells with cold PBS.
3. Resuspend the cell pellet in 150 µL of Tris Buffer (or other general sample buffer) on ice.
4. Homogenize cells quickly by pipetting up and down a few times.

5. Sonicate cells for 50 seconds 3X at high setup (one cycle = 30 s sonication – 10 s break – 10 s sonication – 10 s break)
6. Centrifuge sample for 15 minutes at 4°C at top speed in a cold microcentrifuge to remove any insoluble material.
7. Collect supernatant and transfer to a clean tube.
8. Keep on ice.

Tissue samples:

1. Harvest the necessary amount of tissue necessary for each assay (initial recommendation = 10-30 mg tissue)
2. Wash tissue in cold PBS.
3. Resuspend tissue in 1 mL of Tris Buffer (or other general sample buffer).
4. Add glass beads to sample and homogenize for 1.5 min in the homogenizer. Repeat homogenization step. If using Dounce homogenizer, homogenize with 10 – 15 passes sitting on ice.
5. Centrifuge samples for 15 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.

Serum, plasma and other liquid samples:

Serum and urine samples can be tested directly by adding sample to the microplate wells. No sample pre-treatment is necessary.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (initial recommendation = 1:100 – 1:2,000).

NOTE: We suggest using different volumes of sample to ensure readings are within the Standard Curve range.

Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

1. Plate loading:
   - Standard wells = 200 µL standard dilutions.
   - Sample wells = 1 – 200 µL samples (adjust volume to 200 µL/well with ddH_2O).
2. To prepare the Reaction Mix add 30 µL of Phosphate Reagent to standard and sample wells and mix well.
3. Plate measurement: Incubate plate for 30 minutes at room temperature protected from light. Measure output on a microplate reader at OD 650 nm.
   NOTE: the color is stable for several hours.

Optional protocol for 1 mL cuvettes

Increase all reaction component 5X when using 1 mL cuvettes.

Total reaction mixture (1 mL) will contain:

- 0 – 25 nmol phosphate (0 – 500 µL)
- 150 µL Phosphate reagent

Top up volume to 1.0 mL with ddH_2O

Mix well and incubate at room temperature for 30 minutes protected from light.

Measure output at OD 650 nm on a cuvette spectrophotometer.

Calculations

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean absorbance value of the blank standard (Standard #1) from all standard and sample readings. This is the corrected absorbance.
3. Plot standard curve readings and draw the line of the best fit to construct the standard curve. Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit.
4. Concentration of phosphate in the test samples is calculated as:

$$Phosphate\ Concentration\ = \left(\frac{B}{V}\right) * D$$

Where:

B = amount of phosphate in the sample well calculated from standard curve (nmol).

V = original sample volume added into the reaction well (µL).

D = sample dilution factor.

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