Highly sensitive methods for phosphate detection

THREE ASSAY FORMATS ALLOW YOU TO MEASURE PHOSPHATE LEVELS WHEN—AND HOW—YOU WANT.

Numerous enzymes of therapeutic relevance produce inorganic phosphate directly, or through coupled reactions. These potential drug targets include lipid and protein phosphatases, ATPases, GTPases, prenyltransferases, and phosphodiesterases. The very nature of these targets makes high-quality phosphate assays indispensable to drug discovery research. Here we discuss three assay formats (Table 1) designed to meet different phosphate measurement sensitivity needs depending on sample size and type.

For sensitive, miniaturizable measurements

To address the need for sensitive and miniaturizable phosphate detection, Invitrogen offers Phosphate Sensor. Phosphate Sensor is a purified form of recombinant *E. coli* phosphate-binding protein labeled with the fluorophore MDCC, which is sensitive to changes in its environment (Figure 1). Phosphate binding is tight (K_d ~100 nM), enabling detection in the submicromolar range, which is orders of magnitude more sensitive than the commonly used malachite green (Figure 2). Furthermore, because binding of phosphate is rapid, Phosphate Sensor can be used to monitor phosphate formation in real time, unlike malachite green.

For quantitative, versatile measurements

The P_iPer™ Phosphate Assay Kit also detects in the submicromolar range. Based upon the Amplex® Red reagent, the P_iPer™ Kit enables detection by fluorescence or absorbance. This enzyme-coupled assay ultimately relies on the robust 1:1 stoichiometric reaction between the Amplex® Red reagent and H_2O_2 in the presence of horseradish peroxidase (HRP). The nonfluorescent Amplex® Red reagent produces resorufin, a brightly fluorescing, strongly absorbing reaction product that possesses good quantum efficiency and a high extinction coefficient for a truly quantitative measurement of phosphate. Additionally, because of the long-wavelength spectra, there is little interference from the blue or green autofluorescence found in most biological samples.

Table 1—Spectral properties for Invitrogen™ phosphate assays.

Assay	Abs/Ex*	Em†
Phosphate Sensor	419	466
P_iPer™ Phosphate Assay Kit	563	587
EnzChek® Phosphate Assay Kit	360	NA

* Absorbance or fluorescence excitation maxima, in nm. † Emission maxima, in nm. NA = not applicable.

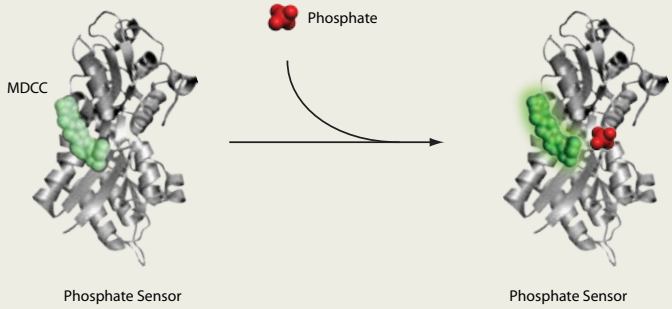


Figure 1—The mechanism behind the Phosphate Sensor reagent. Phosphate Sensor contains a single MDCC fluorophore near the phosphate-binding site. Upon binding phosphate, the fluorescence intensity of the bright blue fluorophore increases dramatically.

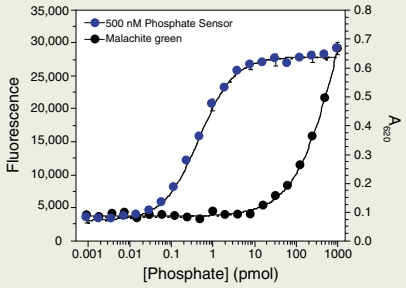


Figure 2—The sensitivity of Phosphate Sensor compared to a malachite green–based reagent. Phosphate standards (10 µl) were added to 384-well plates (clear bottom for malachite green). Either malachite green reagent (70 µl) or Phosphate Sensor (10 µl of 1 µM stock) was added to each well. Absorbance at 620 nm was measured for malachite green. Fluorescence measurements were captured for Phosphate Sensor using a Tecan Safire2™ plate reader with excitation at 430 nm (10 nm bandwidth) and emission at 450 nm (10 nm bandwidth).

For use with more concentrated samples

The absorbance-based EnzChek® Phosphate Assay Kit has an assay range of 2–150 µM, eliminating the need to dilute samples of higher concentration. This assay uses the spectrophotometric shift from 330 nm to 360 nm caused by the phosphorylation of the substrate MESG in the presence of purine nucleoside phosphorylase (PNP) to indicate phosphate levels.

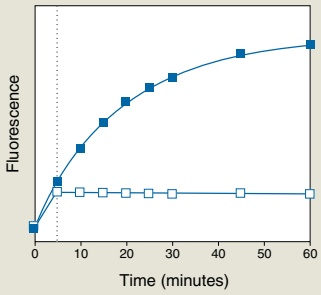
For complete information on Invitrogen's phosphate detection assay portfolio, visit **www.invitrogen.com/phosphate**. ■

PRODUCT HIGHLIGHT

Amplex® Red/UltraRed stop reagent

Stop horseradish peroxidase (HRP) reactions in their tracks. This stop reagent offers unparalleled convenience and control, in addition to:

- compatibility with all Amplex® Red assays and Amplex® Red/UltraRed stand-alone reagents
- a stable fluorescent signal for at least 3 hours
- the ability to terminate reactions containing up to 0.1 units/ml HRP and 5 µM H_2O_2



Application of the Amplex® Red/UltraRed stop reagent to control H_2O_2/peroxidase–coupled detection reactions. Two parallel reactions containing 0.5 mU/ml horseradish peroxidase (HRP) in 50 mM sodium phosphate buffer, pH 7.4, were initiated by addition of 50 µM Amplex® Red reagent + 1 mM H_2O_2. Reaction progress was monitored by detection of the fluorescent product resorufin at 37°C in a fluorescence microplate reader using excitation at 530 ± 12.5 nm and fluorescence detection at 590 ± 17.5 nm. After five minutes (⁘), one of the reactions (□) was terminated by addition of Amplex® Red/UltraRed stop reagent. The fluorescence signal in the stopped reaction remained at the constant level shown for 3 hours (data not shown).

Product	Quantity	Cat. no.
Amplex® Red/UltraRed stop reagent *500 tests* *set of 5 vials*	1 set	A33855

Product	Quantity	Cat. no.
Phosphate Sensor	10 nmol (1,000 assays)	PV4406
Phosphate Sensor	100 nmol (10,000 assays)	PV4407
P_iPer™ Phosphate Assay Kit	1,000 assays	P22061
EnzChek® Phosphate Assay Kit	100 assays	E6646