Store at Room Temp

#12776 Malachite Green Phosphate Detection Kit

1 Kit
(500 assays)

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For Research Use Only. Not For Use In Diagnostic Procedures.

Description: The Malachite Green Phosphate Detection Kit is a convenient and sensitive, single-step free-phosphate determination kit that can be used for measuring phosphate released during enzymatic phosphatase assays.

Background: The Malachite Green Phosphate Detection Kit is designed for the *in vitro* measurement of phosphatase activity. Protein phosphatase enzymatic activity results in the release into solution of free inorganic phosphate from protein-phosphatase substrates. This free inorganic phosphate then forms a green molybdophosphoric acid complex. Formation of this complex is monitored and quantified by measuring the absorbance at 630 nm. This absorbance is proportional to free-inorganic phosphate in solution and correlates with the activity of phosphatase (1,2).

Specificity/Sensitivity: The Malachite Green Phosphate Detection Kit detects only free-phosphate in sample. Figure 1 demonstrates a linear range of 50 to 1600 pmol of phosphate.

Background References:

(1) Baykov, A.A. et al. (1988) *Anal Biochem* 171, 266-70.

(2) Geladopoulos, T.P. et al. (1991) *Anal Biochem* 192, 112-6.

Products Included	Item	Quantity	Storage Temp
Malachite Green Reagent	76120	2 x 28 ml	Room Temp
Phosphate Standard (1 mM)	90943	1 x 0.5 ml	Room Temp

Note: All components in this kit are stable for 12 months when stored at the recommended temperature and left unused.

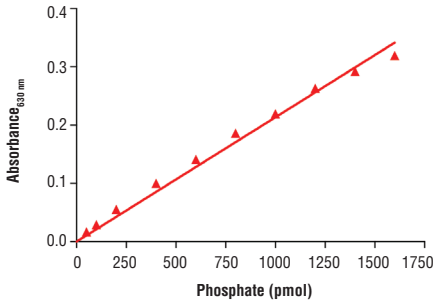

Figure 1. A linear standard curve was generated using phosphate standards and the Malachite Green Phosphate Detection Kit. The phosphate standard was diluted in dH_2O as described in the protocol section and samples were assayed with the Malachite Green Phosphate Detection Kit. This standard curve is for demonstration purposes only; users should generate a standard curve for each sample set in order to accurately determine phosphate concentration.

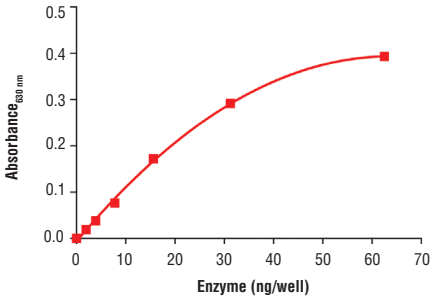

Figure 2. Receptor type protein phosphatase F (PTPRF) activity was measured using the Malachite Green Phosphate Detection Kit. PTPRF phosphatase activity was monitored using tyrosine phosphopeptide-2 (DADE(pY)LIPQQG) as the substrate. The reactions were performed with various amounts of PTPRF phosphatase at 37°C for 10 minutes in a buffer containing 50 mM imidazole, 0.2% 2-mercaptoethanol, 65 ng/µl BSA, and 1 mM tyrosine phosphopeptide-2 (DADE(pY)LIPQQG).

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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Assay Protocol

A. Reagent Included

Malachite Green Reagent 55 ml
Phosphate Standard (1 mM) 0.5 ml

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

B. Creating the Standard Curve

1. Prepare a 0.1 mM working solution of Phosphate Standard by diluting 100 µl of the Phosphate Standard provided (1.0 mM) in 900 µl dH_2O. This solution can be used to prepare a phosphate standard curve as described in the table below (see Table 1). The standard curve should provide a range between 50 and 1600 pmol per well.
2. Transfer 25 µl of each Phosphate Standard titration to a well of a microtiter plate.
3. Add 100 µl of Malachite Green Reagent to each well. Mix carefully without creating any bubbles. (Tip: Use a multichannel pippetor.) The experiment should be performed such that all wells will be read at approximately the same time.
4. Allow color development to proceed for 15-20 min at room temperature.
5. Measure absorbance at a wavelength between 620 and 660 nm. Subtract the absorbance of blank solution (dH_2O only) from standards.
6. Plot pmol of phosphate vs. absorbance for the standard curve.

Table 1.

Volume (µl) of diluted stock (0.1 mM)	160	140	120	100	80	60	40	20	10	5	0
Volume (µl) of distilled water or diluent	90	110	130	150	170	190	210	230	240	245	250
Phosphate [pmol] per 25 µl	1600	1400	1200	1000	800	600	400	200	100	50	0

C. Enzyme Activity Assay

NOTE: Malachite Green is a highly sensitive phosphate detection solution. Make sure any reagent used in the assay is free of inorganic phosphates, as this will greatly increase the background absorbance of the assay. Enzyme reactions should be performed in a final volume of 25 µl (the same volume as that used for the standard curve). Before proceeding, confirm that your enzyme samples are free of contaminating inorganic phosphate. To determine phosphate contamination, add Malachite Green Reagent (100 µl) to 2-5 µl of sample. Any phosphate contamination will result in a color change to green. Measure absorbance at 620-660 nm and compare to standard curve to determine amount of contaminating inorganic phosphate. If required, contaminating inorganic phosphate can be removed using a desalting column or dialysis.

1. Perform phosphatase enzyme reactions in a clean plate with a minimum total reaction volume of 25 µl. Please refer to the manufacture recommended protocols for each phosphatase.
2. Transfer 25 µl of each reaction mixture to individual wells of a microtiter plate.
3. Add 100 µl of Malachite Green Reagent to each well. Mix carefully without creating any bubbles. (Tip: Use a multichannel pippetor.) The experiment should be performed such that all wells will be read at approximately the same time.
4. Allow color development to proceed for 15-20 min at room temperature.
5. Measure absorbance at a wavelength between 620 and 660 nm.

Subtract the absorbance of blank solution.