Standard Range Test Tube Format Phosphate Test Kit

(5.0 to 20.0 ppm Phosphate)

Enzyme and Reagents for 25 Sample Analysis (Including Standards)

Superior Enzymes

5 ppm PO_4 -P = 15.32 ppm PO_4 (ppm PO_4 = (94.97/31) ppm PO_4 -P)

Phosphate-P	1.63 – 6.53 ppm PO₄-P	52.65 – 210.59 µM PO₄	
Phosphate	5.0 - 20.0 ppm PO₄	52.65 – 210.59 µM PO₄	

Purine Nucleoside Phosphorylase (PNP) catalyzes the conversion of the artificial substrate MESG in the presence of inorganic phosphate to release a purine base which absorbs at 360 nm. The purine base that is produced is equimolar to the phosphate, therefore A-360 nm measures phosphate content.

This kit is supplied with enough reagents for 25 total samples, *including the standards*. Please keep this in mind when planning assays.

Supplied in Test Kit:

- MESG in dry powder form- 0.6+ mg 1.5 mL tube in foil pouch
- Purine Nucleoside Phosphorylase (PNP) freeze-dried 1.25 units 1.5 mL tube in foil pouch
- HEPES/MgCl2 buffer in 50mL tube
- **Phosphate Standard (100 ppm Phosphate)** in liquid form 1+ mL
- **Standard tubes** –5 tubes (1.5 mL) for preparing Phosphate Standards
- Dilution tube (5 mL) for preparing stock standard

Supplied by User:

- □ 10 ml graduated cylinder
- **Variable pipettes** (10 to 100 μl and 100 to 1000μl)
- **Test tube vortex-type mixer** or similar to mix contents of tubes
- □ Spectrophotometer capable of reading at 360 nm ± 20 nm, with a UV compatible cuvette (approx. volume 2 ml)
- (25) 13 x 100 mm test tubes (Clean and Phosphate-free)
- **Timer** (0 to 40 minutes) a clock or stop watch is adequate
- □ HPLC H₂O
- Ice and Ice Bucket



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Reagent Preparation

- Step 1 Prepare Assay Buffer Warm the assay buffer to room temperature before use. Store at 4°C between uses - stable for at least 6 months. Store dark.
- Step 2 Prepare 2mM MESG Please follow these instructions carefully
 - 1. Remove tube from foil pouch and tap tube to settle contents. Before opening, make sure the reagent is not stuck to the cap.
 - 2. Add 1 mL of HPLC water.
 - 3. Re-cap and mix well by inversion. Make sure all the powder is in solution.
 - 4. Do not vortex or over-shake.
 - 5. Keep on ice during day of use.

Step 3 Reconstitute PNP – Remove enzyme from foil pouch and tap tube to settle contents. Transfer 1 mL HEPES/MgCl₂ buffer to tube re-cap, and invert tube several times to mix *DO NOT VORTEX*. Let set for at least 5 minutes and mix several more times. Concentration is now 1.25 units/mL.

Step 4 Prepare Reaction Mixture – Prepare fresh each day

- 20.5 mL Assay Buffer (200mM HEPES, 20mM MgCl₂ pH 7.6)
- 1000 μL 2mM MESG
- 1000 μL reconstituted PNP (1.25 units/mL)
- Store reaction mixture in amber (or other light-tight) container. Make sure reaction mixture is room temperature before performing assays.
- Store any unused assay mix at -20°C or colder. It is best to use up all the reaction mixture the same day- only using extra frozen reaction mixture for back-up.



REAGENT NOTES

Assay Buffer – 200mM HEPES, pH 7.6 w/ 20 mM MgCl₂ MESG – 2mM MESG in 200mM HEPES pH 7.6 w/ 20 mM MgCl₂ Purine Nucleoside Phosphorylase (PNP) – 1.25 units/tube Phosphate Standard – 1 vial of 100 ppm Phosphate *Thaw and/or make fresh reagents each day. *For best results, store all unused reagents at -20°C or colder. *Allow reaction mixture to reach room temperature (22°C) before beginning assays.

Standard Preparation

Transfer **1 ml of 100 ppm Phosphate Standard** into 5 mL tube containing **4 mL HPLC water** to make a **20 ppm Phosphate Standard**. Use the snap cap tubes (provided in kit) to prepare Phosphate Standards as shown in table below. Cap and mix the tubes well before use.

Vol 20 ppm PO₄ Standard	Volume HPLC water	Resulting Standard (ppm PO₄)	Resulting Standard (ppm PO₄-P)	Resulting Standard (μM)
-	1.0 mL	0	0	0
0.25 mL	0.75 mL	5	1.63	52.65
0.5 mL	0.5 mL	10	3.26	105.30
0.75 mL	0.25 mL	15	4.90	157.94
1.0 mL	0 mL	20	6.53	210.59

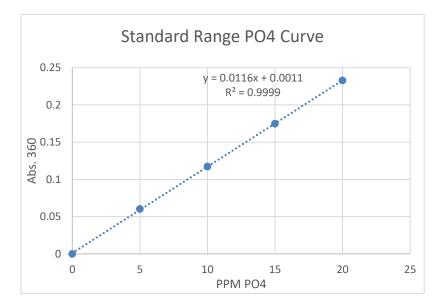


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Phosphate Assay Procedure

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- 1. Pipette 900 µL reaction mixture into each test tube or UV-compatible cuvette.
- 2. Pipette 100 μ L sample, standard, or HPLC H₂O into each tube or cuvette.
- 3. Cap cuvettes and mix by inversion or vortex if using test tubes.
- 4. Incubate for 20 minutes at RT (22°C), mixing reaction tubes every 5 minutes.
- 5. Blank Spectrophotometer with the assay buffer.
- 6. Read samples at A-360 after 20 minute incubation time.
- 7. Create a standard curve. Plot samples against standard curve to get phosphate results.



Example Standard Curve:

