

## Low Range Test Tube Format Phosphate Test Kit

(0.5 to 5.0 ppm Phosphate)



Enzyme and Reagents for 100 Sample Analysis (Including Standards)

$$1 \text{ ppm } PO_4\text{-P} = 3.06 \text{ ppm } PO_4 \text{ (ppm } PO_4 = (94.97/31) \text{ ppm } PO_4\text{-P)}$$

<b>Phosphate-P</b>	<b>0.16 – 1.6 ppm PO<sub>4</sub>-P</b>	<b>5.26 – 52.65 μM PO<sub>4</sub>-P</b>
<b>Phosphate</b>	<b>0.5 - 5.0 ppm PO<sub>4</sub></b>	<b>5.26 – 52.65 μM PO<sub>4</sub></b>

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 100 total samples, *including the standards*. Please keep this in mind when planning assays.

### Supplied in Test Kit:

- MESG** in dry powder form– 3+ mg  
5 mL tube in foil pouch
- Purine Nucleoside Phosphorylase (PNP)** freeze-dried – 25 units  
5 mL tube in foil pouch
- HEPES/MgCl<sub>2</sub>** buffer in (2) 50mL tubes
- Phosphate Standard (100 ppm Phosphate)** in liquid form – 1 mL
- Standard tubes** –7 tubes (5 mL) for preparing Phosphate Standards
- Dilution tube** – (15 mL) for preparing stock standard

### Supplied by User:

- 10 ml graduated cylinder**
- Variable pipettes** (10 to 100 μl and 100 to 1000μl)
- Spectrophotometer** capable of reading at 360 nm, with a UV compatible cuvette (approx. volume 2 ml)
- (100) 13 x 100 mm test tubes** (Clean and Phosphate-free)
- Timer** (0 to 40 minutes) – a clock or stop watch is adequate
- HPLC H<sub>2</sub>O**
- Ice and Ice Bucket**

Clean Water. Fertile Soil. Serious Science.

Support: 906.296.1130 Fax: 906.296.8003 [tech@nitrate.com](mailto:tech@nitrate.com)  
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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 5 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. Aliquot desired volumes and store at -20°C or colder for future use.
6. Keep on ice during day of use.

**Step 3 Reconstitute PNP** – Remove enzyme from foil pouch and tap tube to settle contents. Transfer 5 mL HEPES/ MgCl<sub>2</sub> 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 5 units/mL. Aliquot desired volumes and freeze. (-20°C or colder)  
(1mL of reconstituted enzyme at 5 units/mL performs about 20 assays)

**Step 4 Prepare Reaction Mixture** – Prepare fresh each day (for 19 - 20 assays)

- 8.2 mL Assay Buffer (200mM HEPES, 20mM MgCl<sub>2</sub> – pH 7.6)
- 800 µL 2mM MESG
- 1000 µL reconstituted PNP (5 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. Prepare fresh reaction mixture each day with freshly thawed PNP and MESG, only using extra frozen reaction mixture for back-up.

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**REAGENT NOTES**

**Assay Buffer** – 200 mM HEPES, pH 7.6 w/ 20 mM MgCl<sub>2</sub>

**MESG** – 2 mM MESG in 200mM HEPES pH 7.6 w/ 20 mM MgCl<sub>2</sub>

**Purine Nucleoside Phosphorylase (PNP)** – 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° 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 15 mL tube containing **9 mL HPLC water** to make a **10 ppm Phosphate Standard**. Use the 7 screw cap tubes (provided in kit) to prepare Phosphate Standards as shown in table below. Cap and mix the tubes by inversion before use.

<b>Vol 10 ppm PO<sub>4</sub> Standard</b>	<b>Volume HPLC water</b>	<b>Resulting Standard (ppm PO<sub>4</sub>)</b>	<b>Resulting Standard (ppm PO<sub>4</sub>-P)</b>	<b>Resulting Standard (μM)</b>
-	5 mL	0	0	0
0.25 mL	4.75 mL	0.5	0.16	5.26
0.5 mL	4.5 mL	1.0	0.33	10.53
1.0 mL	4.0 mL	2.0	0.65	21.06
1.5 mL	3.5 mL	3.0	0.98	31.59
2.0 mL	3.0 mL	4.0	1.31	42.12
2.5 mL	2.5 mL	5.0	1.63	52.65

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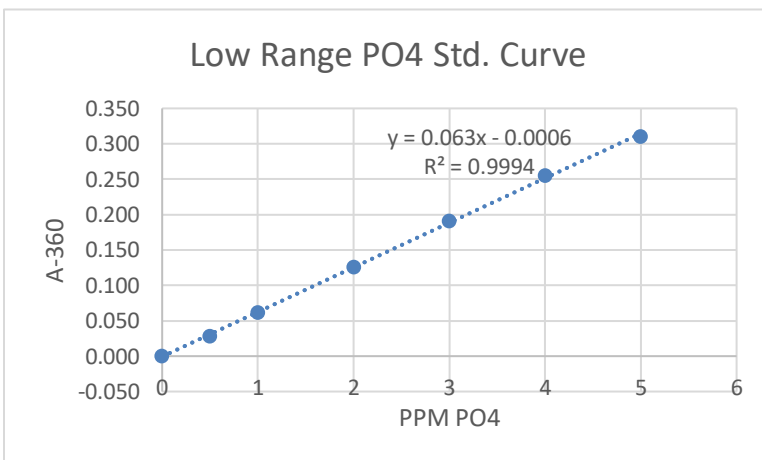
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## Phosphate Assay Procedure

1. Pipette 500  $\mu$ L reaction mixture into each test tube or UV-compatible cuvette.
2. Pipette 500  $\mu$ L sample, standard, or HPLC H<sub>2</sub>O 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:*



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