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 ppm PO_4-P = 3.06 ppm PO_4 (ppm PO_4 = (94.97/31) ppm PO_4-P)

Phosphate-P	**0.16 – 1.6 ppm PO_4-P**	**5.26 – 52.65 µM PO_4-P**
Phosphate	**0.5 - 5.0 ppm PO_4**	**5.26 – 52.65 µM PO_4**

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/$MgCl_2$** 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_2O**
- ❑ **Ice and Ice Bucket**

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/ $MgCl_2$ 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 $MgCl_2$ – 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.

REAGENT NOTES

Assay Buffer – 200 mM HEPES, pH 7.6 w/ 20 mM $MgCl_2$
MESG – 2 mM MESG in 200mM HEPES pH 7.6 w/ 20 mM $MgCl_2$
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.

Vol 10 ppm PO_4 Standard	Volume HPLC water	Resulting Standard (ppm PO_4)	Resulting Standard (ppm PO_4-P)	Resulting Standard (µM)
-	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

Phosphate Assay Procedure

1. Pipette 500 µL reaction mixture into each test tube or UV-compatible cuvette.
2. Pipette 500 µL sample, standard, or HPLC H_2O 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: