Standard Range Test Tube Format Phosphate Test Kit

(5.0 to 20.0 ppm Phosphate)

Enzyme and Reagents for 25 Sample Analysis (Including Standards)

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 PO_4-P**	**52.65 – 210.59 µM PO_4**
Phosphate	**5.0 - 20.0 ppm PO_4**	**52.65 – 210.59 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 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 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 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/$MgCl_2$ 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 1.25 units/mL.

Step 4 **Prepare Reaction Mixture – Prepare fresh each day**

- 20.5 mL Assay Buffer (200mM HEPES, 20mM $MgCl_2$ – 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 $MgCl_2$
MESG – 2mM MESG in 200mM HEPES pH 7.6 w/ 20 mM $MgCl_2$
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 PO_4 Standard	Volume HPLC water	Resulting Standard (ppm PO_4)	Resulting Standard (ppm PO_4-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

Phosphate Assay Procedure

1. Pipette 900 µL reaction mixture into each test tube or UV-compatible cuvette.
2. Pipette 100 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: