

2.5 hrs

# PHOS Test Cheat Sheet

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Usual Set Up:

- RB:** Reagent Blank
- 1-7:** Curve Standards
- 8:** Reference
- 9&10:** Influent Day 1
- 11&12:** Secondary Day 1
- 13&14:** Effluent Day 1
- 15:** Effluent Day 1 + Spike
- 16&17:** Influent Day 2
- 18&19:** Secondary Day 2
- 20&21:** Effluent Day 2
- 22:** Effluent Day 2 + Spike
- 23&24:** Complex Nutrient Standard
- 25:** Blank

RB					
1	2	3	4	5	6
7	8				
9	10			11	12
13	14	15			

16	17			18	19
20	21	22			
23	24				25

Steps:

- 1) ADD 5000 uL reagent water to ALL TUBES using dispensette.
- 2) ADD appropriate volumes of water using pipettes (or Eppendorf repeater) to EACH TUBE as follows (uL):  
 \*note: it's helpful to do all of the tubes with the same volumes together (i.e. RB, 1-8, 25. Then 9, 10, 16, 17, 23, 24. Etc.)

RB 3000					
1 3000	2 3000	3 3000	4 3000	5 3000	6 3000
7 3000	8 3000				
9 2500	10 2500			11 1000	12 1000
13 0	14 0	15 0			

16 2500	17 2500			18 1000	19 1000
20 0	21 0	22 0			
23 2500	24 2500				25 3000

- 3) REMOVE specified amounts of water and ADD SAME AMOUNT of the following ingredients (uL) to each tube as follows: (\*note: helpful to add/subtract ingredients from tubes 4, 8, 15, 22 at same time to reduce volume error)

*nirse*

- a. Phos standards (ampoule) to each of tubes 2-7
- b. Phos reference (bottle) to tube 8
- c. Spike (ampoule) to tubes 15 & 22

*alixard  
HP*

RB					
1	2	3	4	5	6
	-0.8 +0.8	-8.0 +8.0	-12.0 +12.0	-16.0 +16.0	-20.0 +20.0
7	8				
-24 +24	-12 +12				
9	10			11	12
13	14	15			
		-12 +12			

16	17			18	19
20	21	22			
		-12 +12			
23	24				25

4) **ADD** samples and complex nutrients to specified tubes as follows (uL) using pipette:

- Samples to respective tubes 9-22
- Complex nutrients to tubes 23 & 24

*shake in vortex(?)*

\*\*\*all tubes should have 8mL total solution after this

RB					
1	2	3	4	5	6
7	8				
9 500	10 500			11 2000	12 2000
13 3000	14 3000	15 3000			

16 500	17 500			18 2000	19 2000
20 3000	21 3000	22 3000			
23 500	24 500				25

- 5) Add 160 uL of 35% sulfuric acid to **ALL TUBES** (using pipette or Eppendorf repeater).
- 6) Add 0.5g potassium persulfate to **ALL TUBES EXCEPT BLANK!** *RB*
- 7) Cap & Seal. Invert to mix. *RB*
- 8) Autoclave all tubes **EXCEPT BLANK** (Erlenmeyer flask button- 30 min). Leave 10 min to cool.
- 9) Wash tubes using kim wipes and reagent water.
- 10) Add 640 uL Ammonium molybdate to **ALL TUBES** (using pipette or Eppendorf repeater).
- 11) Add 320 uL Ascorbic acid to **ALL TUBES** (using pipette or Eppendorf repeater).
- 12) Cap & Seal. Invert to mix. Let sit undisturbed for 5 min.
- 13) Take absorbance values using spectrophotometer at 660nm wavelength. Record on sheet.

*wipe caps*

**\*\*Note:** Ascorbic Acid has a shelf life of 2 weeks and will need to be remade often, regardless of how much is left in bottle:

-Fill round 100mL mixing flask ¾ full with Millipore water. Measure out 6 grams of Ascorbic Acid. Add ascorbic acid and 200 uL of acetone to flask. Mix well. Add water to 100 mL line. Mix well. Pour solution into [empty and rinsed] ascorbic acid storage bottle.