

	Sequence steps	Volumes/flowrates
Activate instrument	Turn on analyzer Turn light off Get dark scan (see seabreeze code) Turn lamp on Wait 3 minutes for lamp to warm up	
Run Blank (2x)	Get reference scan (sea seabreeze code) Pump 1 dispense COV-6 go to port 3 (Molybdate reagent) Pump 2 aspirate Pump 1 dispense Wait 2 seconds COV-6 go to port 5 (Ascorbic acid reagent) Pump 1 aspirate Pump 2 dispense Wait 2 seconds Go to port 2 (flow cell) Pump 1 dispense Wait 5 minutes Get absorbance value of blank sample (see seabreeze code) Pump 1 dispense Pump 2 dispense	volume (μL), flow rate (μL/s), hold all (0/1 600,60,1 volume (μL), flow rate (μL/s), hold all (0/1 400,40,0 volume (μL), flow rate (μL/s), hold all (0/1 320,32,1 volume (μL), flow rate (μL/s), hold all (0/1 400,40,0 volume (μL), flow rate (μL/s), hold all (0/1 320,32,1 volume (μL), flow rate (μL/s), hold all (0/1 400,25,1 volume (μL), flow rate (μL/s), hold all (0/1 1000,150,0 volume (μL), flow rate (μL/s), hold all (0/1 1000,150,1
Run PO4	Get reference scan (sea seabreeze code) COV-6 go to port 6 (PO4 standard) Pump 1 aspirate COV-6 go to port 3 (Molybdate reagent) Pump 2 aspirate Pump 1 dispense Wait 2 seconds COV-6 go to port 5 (Ascorbic acid reagent) Pump 1 aspirate Pump 2 dispense Wait 2 seconds	volume (μL), flow rate (μL/s), hold all (0/1 600,60,1 volume (μL), flow rate (μL/s), hold all (0/1 400,40,0 volume (μL), flow rate (μL/s), hold all (0/1 320,32,1 volume (μL), flow rate (μL/s), hold all (0/1 400,40,0 volume (μL), flow rate (μL/s), hold all (0/1 320,32,1

standards (2x)	Go to port 2 (flow cell)	
	Pump 1 dispense	volume (μL), flow rate (μL/s), hold all (0/1 400,25,1
	Wait 5 minutes	
	Get absorbance value of PO4 standard (see seabreeze code)	
	COV-6 go to port 3 (Molybdate reagent)	
	Pump 1 aspirate	volume (μL), flow rate (μL/s), hold all (0/1 150,50,1
	COV-6 go to port 6 (PO4 standard)	
	Pump 1 aspirate	volume (μL), flow rate (μL/s), hold all (0/1 150,50,1
	Go to port 2 (flow cell)	
	Pump 1 dispense	volume (μL), flow rate (μL/s), hold all (0/1 1000,150,0
	Pump 2 dispense	volume (μL), flow rate (μL/s), hold all (0/1 1000,150,1
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	Get reference scan (see seabreeze code)	
	Run auxiliary pump for 60 seconds	
	COV-6 go to port 4 (PO4 sample)	
	Pump 1 aspirate	volume (μL), flow rate (μL/s), hold all (0/1 600,60,1
	COV-6 go to port 3 (Molybdate reagent)	
	Pump 2 aspirate	volume (μL), flow rate (μL/s), hold all (0/1 400,40,0
	Pump 1 dispense	volume (μL), flow rate (μL/s), hold all (0/1 320,32,1
	Wait 2 seconds	
	COV-6 go to port 5 (Ascorbic acid reagent)	
Run PO4 seawater sample (1x)	Pump 1 aspirate	volume (μL), flow rate (μL/s), hold all (0/1 400,40,0
	Pump 2 dispense	volume (μL), flow rate (μL/s), hold all (0/1 320,32,1
	Wait 2 seconds	
	Go to port 2 (flow cell)	
	Pump 1 dispense	volume (μL), flow rate (μL/s), hold all (0/1 400,25,1
	Wait 5 minutes	
	Get absorbance value of PO4 sample (see seabreeze code)	
	COV-6 go to port 3 (Molybdate reagent)	
	Pump 1 aspirate	volume (μL), flow rate (μL/s), hold all (0/1 150,50,1
	COV-6 go to port 4 (PO4 sample)	
	Pump 1 aspirate	volume (μL), flow rate (μL/s), hold all (0/1 150,50,1
	Go to port 2 (flow cell)	
	Pump 1 dispense	volume (μL), flow rate (μL/s), hold all (0/1 1000,150,0

Pump 2 dispense

volume (μL), flow rate ($\mu\text{L/s}$), hold all (0/1 1000,150,1

Calculate PO4 concentration:

$[\text{PO}_4] = (\text{Abs_sample} - \text{mean_Abs_blank}) \times [\text{concentration of po4 standard}] / (\text{mean_Abs_standard} - \text{mean_Abs_blank})$

post-processing Store sample time stamp + sample concentration, send to server

Sleep until next sample (~40 min if wanting hourly measurements)

Notes

Dispensing blank sample (carrier)

Pumps run simulatenously

Pumps run simulatenously

***depends on flow cell length (used 300 with 10cm FC)

**Could be shortened given no PO4 in blank

Pumps run simultaneoslty to flush instrument

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***depends on flow cell length (used 300 with 10cm FC)

Priming step: added 4/4/22

Pumps run simultaneously to flush instrument

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