Method-No. G-175-96 Rev. 13 (Multitest MT 18)

Phosphate in Water and Seawater
Total P in persulfate or Kjeldahl digests

Ranges: 0 – 4.8 to 0 - 61 μmol/L (0 – 0.15 to 0 – 1.9 mg/L as P) *
and 0 - 55 to 0 - 550 μmol/L (0 – 1.7 to 0 – 17 mg/L as P)

Description

Following the method of Murphy and Riley, this automated procedure for the determination of ortho-phosphate is based on the colorimetric method in which a blue color is formed by the reaction of ortho-phosphate, molybdate ion and antimony ion followed by reduction with ascorbic acid at an pH<1. The reduced blue phospho-molybdenum complex is read at 880 nm. The [H+] : [Mo] ratio in the reaction mixture corresponds to the optimum determined by Riley and Yang. The method is also applicable to samples digested with alkaline persulfate and, in the high range and with different reagents, to Kjeldahl digests.

* For low level acid Kjeldahl digests (2 – 1200 μg/L as P) see operating note 12

Hardware: 37°C heating bath (5.37 mL) **Pump tubes**: 5 + 2 air + sampler wash
Multitest: Nitrite, Phenol and Total Phosphorus in Kjeldahl digests

Performance data using aqueous standards and AA3 colorimeter

Test conditions: range: 0 - 26 and 0 - 260 μmol/L and AA3 colorimeter with 10 mm flowcell and lamp

	Sample A 0 - 26 μmol/L	**Sample B 0 - 260 μmol/L**
Pump tube	blu/blu	orn/yel
Sampling rate	60/h	60/h
Sample : wash ratio	4:1	4:1
Sensitivity: Extinction at 26 / 260 μmol/L	0.40-0.44	0.42-0.46
Reagent absorbance	0.02-0.04	0.02-0.04
Coefficient of variation (10 replicates at 50%)	0.2%	0.2%
Pooled standard deviation (25 randomised at 5 levels)	0.012 μmol/L	0.12 μmol/L
Correlation Coefficient (linear, 5 points)	0.9999	0.9996
Detection limit (determined according to EPA procedure pt. 136, app. B)	0.024 μMol/L	
Detection limit in lowest range (lowest range 0 - 6.5 μmol/L)	0.020 μMol/L	

Note: the above performance specifications were obtained with the exclusive use of genuine SEAL Analytical parts and consumables.

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REAGENTS

Unless otherwise stated all chemicals should be of Analytical Reagent grade or equivalent (e.g. ACS grade, Analar, Pro Analysi).

LIST OF RAW MATERIALS

	safety classification
Acetone, C_3H_6O	flammable
Ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$	harmful
Antimony potassium tartrate, $K(SbO)C_4H_4O_6.1/2H_2O$	toxic
Ascorbic acid, $C_6H_8O_6$	--
Potassium dihydrogen phosphate, KH_2PO_4	--
Sodium chloride, NaCl	--
Sodium hydrogen carbonate, $NaHCO_3$	--
Sodium dodecyl sulfate, SDS (ultra-pure grade required)	harmful
Sodium hydroxide, NaOH (see operating note 12)	corrosive
Sulfuric acid, H_2SO_4	corrosive
Low-nutrient seawater: see operating note 2	

REAGENT MAKE UP

Prepare reagents with distilled water or deionized water. Vacuum filter reagents through filter with pore size 0.5 µm or less for best results.

SYNTHETIC SEAWATER

(see operating notes 1 and 2)

Sodium chloride	35 g
Sodium hydrogen carbonate	0.2 g
DI water	to 1000 mL

Dissolve 35 g of sodium chloride and 0.2 g of sodium hydrogen carbonate in about 800 mL of DI water. Dilute to 1000 mL with DI water and mix thoroughly.

SYSTEM WASH SOLUTION

Use DI Water containing 8 g/L SDS.

SPECIAL WASH SOLUTION

Use sodium hypochlorite solution diluted 1:5 with DI water.

STOCK ANTIMONY POTASSIUM TARTRATE

Antimony potassium tartrate	2.3 g
DI water	to 100 mL

Dissolve 2.3 g of antimony potassium tartrate in about 80 mL of DI water. Dilute to 100 mL with DI water and mix thoroughly. The solution is stable for a month.

AMMONIUM MOLYBDATE
(see operating note 12)

Ammonium molybdate	6 g
Sulfuric acid, conc.	64 mL
Stock antimony potassium tartrate	22 mL
DI water	to 1000 mL

Add carefully 64 mL of conc. sulfuric acid to about 500 mL of DI water and cool. Dissolve 6 g of ammonium molybdate and add 22 mL of stock antimony potassium tartrate. Dilute to 1000 mL with DI water and mix thoroughly. Store in a dark bottle. The solution is stable for a month. The solution must be colourless. The ammonium molybdate must be perfectly white, with no green tint.

ASCORBIC ACID
(see operating note 11)

Ascorbic acid	8 g
Acetone	45 mL
Sodium dodecyl sulphate (SDS)	8 g
DI water	to 1000 mL

Dissolve 8 g of ascorbic acid in about 600 mL of DI water. Add 45 mL of acetone and 8 g of sodium dodecyl sulphate. Dilute to 1000 mL with DI water and mix thoroughly. Store in a dark bottle in the refrigerator. The solution is stable for 1 week. Ultra-pure SDS is critical to good method performance.

SODIUM HYDROXIDE
(see operating note 12)

Sodium hydroxide	4.6 g
DI water	to 1000 mL

Dissolve 4.6 g of sodium hydroxide in about 600 mL of DI water. Cool down to room temperature. Dilue to 1000 mL and mix thoroughly. Store in a plastic bottle. Stable as long as the solution remains clear.

STANDARDS

STOCK STANDARD A, 500 mg/L

Potassium dihydrogen phosphate	2.197 g
DI water	to 1000 mL

Dry 2.5 g of potassium dihydrogen phosphate at 105°C for 2 hours. Dissolve 2.197 g of potassium dihydrogen phosphate in about 600 mL of DI water. Dilute to 1000 mL with DI water and mix thoroughly.

STOCK STANDARD B1, 10 mg/L

Stock standard A	2 mL
DI water	to 100 mL

Dilute 2 mL of Stock Standard A in a 100 volumetric flask. Dilute to 100 mL with DI water and mix thoroughly.

STOCK STANDARD B2, 5 mg/L

Stock standard A	1 mL
DI water	to 100 mL

Dilute 1 mL of Stock Standard A in a 100 volumetric flask. Dilute to 100 mL with DI water and mix thoroughly.

WORKING STANDARDS

Prepare working standards as required. Use synthetic seawater for seawater analysis. If analyzing Total P, standards must be digested and diluted in the same way as samples (see operating note 2, 10 and 12).

OPERATING NOTES

1. **Sampler Wash.** For water and wastewater analysis use DI water as the sampler wash solution. For seawater analysis it is possible to use containing sodium chloride and sodium hydrogen carbonate or DI water. If using DI water, make sure the peak window is set to read only the peak plateau. It may be useful to increase the sampling time by up to 25 seconds. For Kjeldahl digests use diluted H_2SO_4 at the same concentration as the samples (see operating note 10 and 12).

2. The diluent used for standards must have the same matrix as the samples. Therefore, use artificial seawater or low-nutrient seawater for seawater analysis. To avoid errors from phosphate content in the inorganic salts used for artificial seawater, we recommend using a zero calibration standard of low-nutrient seawater of known low concentration. This is obtainable from Ocean Scientific International, Station Road, Petersfield, Hampshire, England GU32 3ET. Fax +44 1730 265011. For Total P analysis, the standards should be digested using the same procedure as for the samples.

3. **Recommended procedures for best performance when analyzing low concentrations**

   - Pure water may be double distilled (DD) water or deionized (DI) water. In the case of DDW, the analyst must be careful to avoid contamination with silicic acid from dissolution of glass.
   - For accurate low-level work, all glassware used for making reagents should be rinsed with 10% hydrochloric acid followed by thorough rinsing with DI water two or more times. Store flasks “shaken dry” and capped. Regular cleaning of storage containers reduces variances in analytical results. Do not wash the glassware in a washer or with any kind of detergent.
   - Sample cups must be perfectly clean. For low-level work, fill sample cups with 10% hydrochloric acid and leave standing for at least 15 min. Then rinse the sample cups twice with DI water followed by two rinses with sample or standard solution.
   - Sample storage or transport containers may be made of any of several plastics. High density polyethylene or polypropylene bottles are very acceptable. Glass containers of any kind are not acceptable. Any glass contaminates the samples with silicic acid. Sample containers must be rinsed at least twice with sample before filling.
   - Skin contact must be avoided with anything which will touch the reagents and samples. Ammonia contamination of the air must be avoided (e.g. by smoking, farmyard, industrial smoke or vapour, other reagents).
   - The laboratory temperature should be reasonably stable, with no strong air currents around analyzer. Run the system with the manifold cover in place.
   - All chemicals should be of very high purity. Old and/or contaminated SDS will cause carryover, drift and noise. Final working standards are best prepared using natural artificial seawater of low nutrient content (see operating note 2).
   - The prepared reagents should be degassed by vacuum membrane filtration for best performance. Filter with a pore size of 0.5 µm or less should be used. The reagents, pure water and standards should be protected from atmospheric contamination.
   - Samples should be measured as soon as possible after sampling.

- Rinse the manifold according to operating note 4. Rinse wash receptacle each day by pumping baseline reagents for 15 minutes before starting a run. Clean the wash receptacle once a month with hypochlorite solution.
- The volume between the air valve and the injection fitting should be minimal, using 0.015" polyethylene tubing cut as short as possible. The joints between glass parts must be perfect without gaps.
- If running only in the lowest range the baseline noise can be reduced by diluting the reagents by a factor of 2 or even 5. The linearity of the used range must be checked.
- A regular bubble pattern is necessary for low noise. If the bubble pattern is irregular, check that all plastic tubing is correctly wetted (bubble shape round at front and back. After replacing the pump tubes or parts of the manifold, pump 1M NaOH through all tubes for 15 minutes. (see also operating note 11).

4. **Manifold cleaning procedure:**
   Every day ⇨ pump system wash solution (8 g/L SDS) through all reagent lines.
   Once a month ⇨ pump for 20 min. special wash solution (hypochlorite) through the system and the sample line, then 30 min. system wash solution.

5. If the high range is not needed, remove sample line B and tie off or remove the T-piece.

6. Pump DI water through the sample line which is not connected to the sample probe.

7. The connection between the cartridge and the colorimeter should be made of glass in order to reduce the carryover.

8. If the ortho-phosphate chemistry is to be used following a chemistry that uses Brij-35 as wetting agent (e.g. nitrite), wash thoroughly with 1 N H_2SO_4 for 10 minutes before pumping wash solution for 15 minutes and then connecting the reagents.

9. **Additional performance data:**

Lag time	5 - 7 min.
Carryover	0.2%

10. For **total P by persulfate digestion**, prepare the digestion reagent according to the procedure of K. Grasshoff et. al. (Methods of Seawater Analysis, 2nd Edition, Verlag Chemie, 1983) as follows:

    15g H_3BO_3, 25g $K_2S_2O_8$, 7.5g NaOH diluted to 500 mL with water.

    Prepare the digestion reagent fresh weekly.
    To digest samples, add 5 mL reagent to 50 mL sample in a suitable PTFE pressure bottle. Heat at 115ºC for 2 hours. The pH of the digested samples should be about 8.

11. Even flow and regular air/liquid distribution in the transmission tube from the debubbler after the first mixing coil to the pump is critical to correct method performance. Check for correct flow and that the tubing is wetted (trailing edge of the bubbles must be rounded, not straight). If necessary, especially for new tubing, increase the concentration of surfactant to achieve correct wetting. See also (14).

12. When analyzing diluted **Kjeldahl digests**, adjust the acid concentration depending on the dilution of the digests. When diluted to the recommended ratio of 4% sulfuric acid by volume in the digests, no acid is required in the molybdate reagent. Samples at 2% acid will require 32 ml acid in the reagent. Samples at higher acid content may be run, but the sensitivity will be reduced. Kjeldahl digests in high ranges can be normally analyzed through the high-level sample line ("sample B").

    When analyzing **acid Kjeldahl digests in low level range** (2 – 1000 μg/L as P) perform the following modifications:

    - Change pumb tube "sample B" from orn/yel (0.16) to wht/wht (0.60)

    - Run NaOH solution through line "sample A"

- The method is linear up to a range from 2 – 1200 µg/L. For higher ranges use a quadratic fit.

13. LED Photometer
By the operation of the AA3 on research vessels it is recommended to use the LED photometer. The noise of the signal caused by vibration and movement of the ship is reduced compared to the lamp photometer. The special filter for the LED must be used. The filter from the lamp photometer can not be used for the LED. The performance data may change slightly by the use of the LED photometer.

14. If the bubble pattern out of the heating bath becomes irregular the size of the second air injection pump tube may be increased from blk/blk to orn/orn.

REVISIONS

Revision 1, February 1999
Added AA3 data; integrated flow diagram

Revision 2, April 1999
p/n numbers of tubes (used in colorimeter) added to flow diagram and parts list

Revision 3, July 2000
Corrected make-up of standard, recalculated performance data, added persulfate digest information.

Revision 4, March 2001
Added operating note 13. Corrected error in detection limit figures in µg/L.

Revision 5, April 2001
Added new glassware for AA3 colorimeter

Revision 6, August 2001
Expanded notes, added range and performance data for AA3 system to first page, removed AAII performance data.

Revision 7, November 2001
Expanded notes, slight text changes in preparation of stock solution.

Revision 8, February 2002
Added LED photometer, expanded notes

Revision 9, May 2002
Correction of the flowchart

Revision 10, December 2002
Changed pump tube air to blk/blk (0.32)

Revision 11, March 2003
Low range Kjeldahl digests: modifed operating note 12, added NaOH solution

Revision 12, March 2005
Added Note 14.

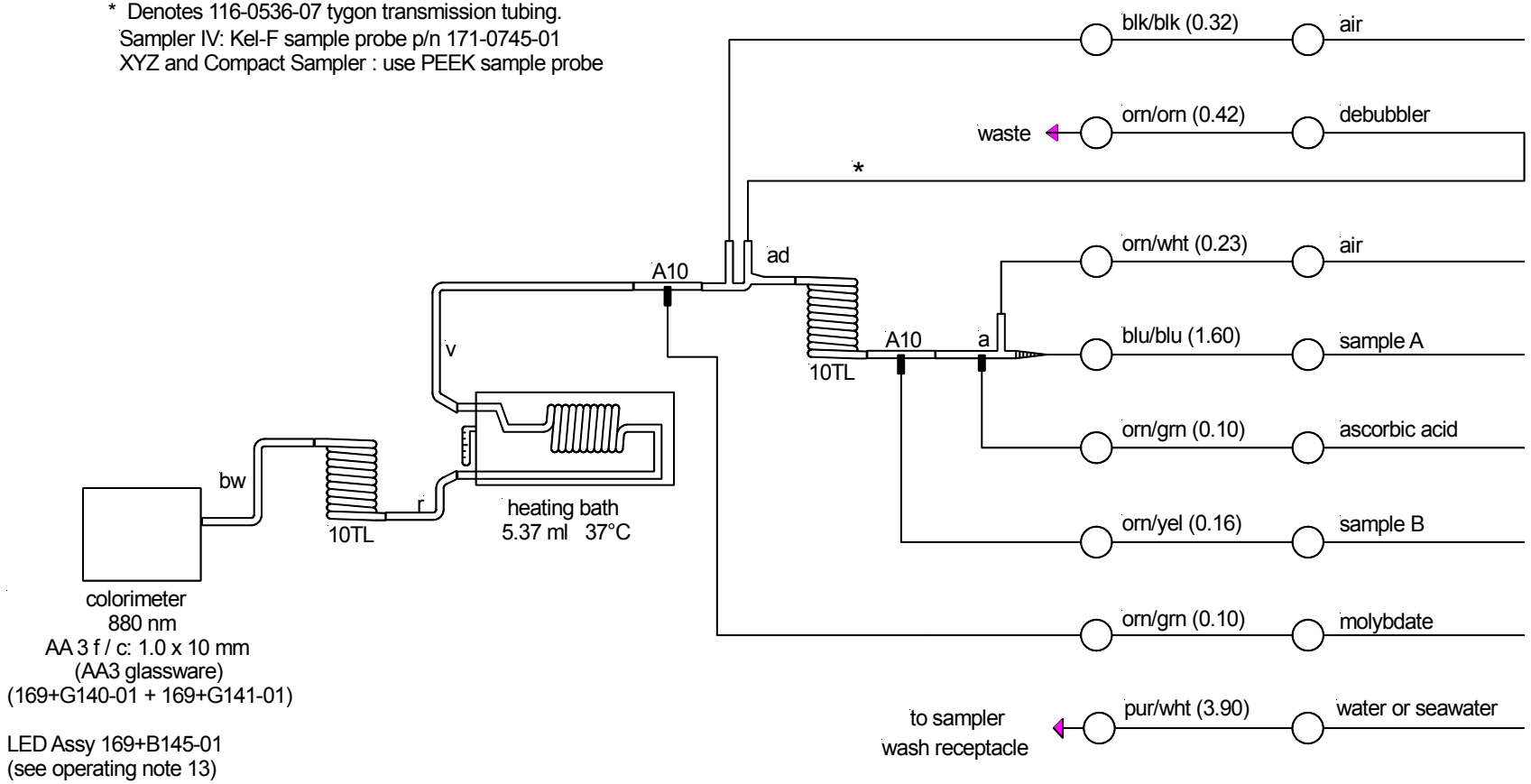
Revision 13, April 2008
Changed logo

CONSUMABLES

The following estimated annual consumption rates are based on system operation 8 hours/day, 250 days/year.

Description	Legend	Part Number	Est. Annual Usage
BLK/BLK, 0.32 mL/min		116-0549-07	2 pkg./12
ORN/GRN, 0.10 mL/min		116-0549-04	2 pkg./12
ORN/YEL, 0.16 mL/min		116-0549-05	1 pkg./12
ORN/WHT, 0.23 mL/min		116-0549-06	2 pkg./12
ORN/ORN, 0.42 mL/min		116-0549-08	1 pkg./12
WHT/WHT, 0.60 mL/min		116-0549-09	1 pkg./12 (see operation note 12)
YEL/BLU, 1.40 mL/min		116-0549-19	1 pkg./12
BLU/BLU, 1.60 mL/min		116-0549-13	1 pkg./12
PUR/WHT, 3.90 mL/min		116-0549-18	1 pkg./12
Tubing air bar PharMed		117+0539-07	1 m
Polyethylene tubing 0.015” ID		562-2002-01	1 m
Polyethylene tubing 0.03" ID	*	562-2015-01	1 m
Tygon tubing		116-0536-07	1 m
Tygon tubing		116-0536-11	1 m
Tygon tubing		116-0536-16	1 m
Sample cups, plastic, 5 mL		171-0354-01	1 pkg./1000
Sample cups, plastic, 4 mL		127-0018-01	1 pkg./1000
Sample tubes, plastic, 8 mL		168-1000-01	1 pkg./2000
Sample tubes, plastic, 11 mL		168-1001-01	1 pkg./1600
Tubes for standards, 15.5 mL (XY2/3)		168-1004-01	1 pkg./100
SPARES			Recommended holding
AA3 Flowcell, 1.0 x 10 mm		169+B040-10	1 pc.
AA3 Colorimeter lamp		169+B143-01	1 pc.
AA3 Filter Assy, 880 nm		165+B044-88	1 pc.
LED Assy, 880 nm		169+B145-01	1 pc.
AA3 colorimeter glassware		169+G140-01	1 pc.
AA3 colorimeter glassware		169+G141-01	1 pc.
Injection fitting, 3 pt.	a	116-0489-01	1 pc.
Glass coil, 10 turns left	10TL	157-0226-01	2 pcs.
AA3 Coil, 5.37 mL		169+B441-01	1 pc.
AA3 Heater assembly		169+B410-01	1 pc.
AA3 Controller, 115V/230V		169+B430-01/02	1 pc.
Thermometer 32-42°C		157-0283-01	1 pc.
Glass tubing	r	170-0193-01	1 pc.
Glass tubing	v	170-G014-01	1 pc.
Separator-phase 4 pt.	ad	021-G001-01	1 pc.
Glass tubing	bw	194-G003-05	1 pc.
Connector T	A10	116-B034-01	2 pcs.

NOTES : Figures in parentheses are flowrates in ml/min.
* Denotes 116-0536-07 tygon transmission tubing.
Sampler IV: Kel-F sample probe p/n 171-0745-01
XYZ and Compact Sampler : use PEEK sample probe



DRAWN	S.Giedigkeit	01.03.99	SYSTEM	AA3	PARAMETER	Phosphate
CHANGED	S.Giedigkeit	24.04.2008	METHOD NO. (11)	G-175-96	MATRIX	Water and seawater
RELEASED	A.Jorissen	24.04.2008	REMARK	Multitest MT18	RANGE	Sample A:0-4.8 to 0-81 µmol/L as P Sample B:0-55 to 0-550 µmol/L as P

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