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# AutoAnalyzer Applications

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Method No. G-172-96 Rev. 12 (Multitest MT 19)

## Nitrate and Nitrite in Water and Seawater Total Nitrogen in persulfate digests

**Ranges: 0 - 2.9 to 0 – 46  $\mu\text{mol/L}$  (0 - 40 to 0 - 650  $\mu\text{g/L}$  as N)  
and 0 - 50 to 0 - 500  $\mu\text{mol/L}$  (0 - 700 to 0 - 7000  $\mu\text{g/L}$  as N)**

### Description

This automated procedure for the determination of nitrate and nitrite uses the procedure whereby nitrate is reduced to nitrite by a copper-cadmium reductor column (1,2). The nitrite then reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-naphthylethylene diamine dihydrochloride to form a purple azo dye. In most normal water and seawater samples the concentration of oxidising or reducing agents and interfering metal ions is well below the limits causing interferences. When present in sufficient concentration, metal ions may produce a positive error, i.e. divalent mercury and divalent copper may form coloured complex ions having adsorption bands in the region of the colour measurement (3). Significant amounts of sulfate, sulfide or organic material, especially oil, interfere with the performance of the copper-cadmium reductor column. Such samples should be pre-treated before analysis. The method is also suitable for persulfate digested samples (see operating note 15) (4). The method is based on the nitrate determination in Standard Methods and in the DIN / ISO Standards for automatic nitrate measurements.

**Hardware:** 37°C heating bath (5.4 mL), Cd column      **Pump tubes:** 6 + 2 air + sampler wash  
**Multitest:** aluminium, ammonia, colour, copper, chloride, iron, manganese, nitrate, total N in persulphate digests, nitrogen (total Kjeldahl), phosphate, total phosphorus (Kjeldahl), silicate, sulphide and zinc.

### Typical performance using aqueous standards and AA3 colorimeter

Test conditions: range: 0 - 21  $\mu\text{mol/L}$  and 0 – 285  $\mu\text{mol/L}$  with AA3 colorimeter (10 mm flowcell and lamp)

	Sample A 0 - 21 $\mu\text{mol/L}$	Sample B 0 - 285 $\mu\text{mol/L}$
Pump tube	yel/yel	orn/grn
Sampling rate	60/hr	60/hr
Sample: wash ratio	4:1	4:1
Sensitivity: Extinction at 21 / 285 $\mu\text{mol/L}$	0.44-0.48	0.53-0.57
Reagent Absorbance	0.01-0.03	0.01-0.03
Coefficient of Variation (10 replicates at 50 %)	0.21 %	
Pooled Standard Deviation (25 randomised at 5 levels)	0.022 $\mu\text{mol/L}$	
Correlation Coefficient (5 points, linear)	0.999	
Detection Limit (determined according to EPA procedure pt. 136, app. B)	0.015 $\mu\text{mol/L}$	
Detection Limit in lowest range (lowest range 0- 2.9 $\mu\text{mol/L}$ )	0.010 $\mu\text{mol/L}$	

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

### References

1. Armstrong, F.A.J., Sterns, C.R. and Strickland, J.D.H., 1967 Deep Sea Res. 14, pp. 381-389. "The measurement of upwelling and subsequent biological processes by means of the Technicon AutoAnalyzer and associated equipment."
2. Grasshoff, K., Technicon International Congress, June 1969.
3. Federal Water Pollution Control Administration Methods for Chemical Analysis of Water and Wastes, November 1969.
4. Methods of Seawater Analysis, K. Grasshoff et. al., Verlag Chemie, 2<sup>nd</sup> Edition, 1983.



## REAGENTS

Unless otherwise specified all chemicals should be of analytical grade or equivalent.

### LIST OF RAW MATERIALS

	<i>safety classification</i>
Ammonium chloride, NH <sub>4</sub> Cl	--
Ammonia solution, 25% NH <sub>3</sub>	harmful
Brij-35, 30% solution	--
Cadmium, coarse powder; particle size 0.3-0.8 mm	toxic
Hydrochloric acid, conc., HCl	corrosive
N-1-Naphthylethylenediamine dihydrochloride, C <sub>12</sub> H <sub>14</sub> N <sub>2</sub> .2HCl	--
Phosphoric acid, conc., H <sub>3</sub> PO <sub>4</sub>	corrosive
Potassium nitrate, KNO <sub>3</sub>	oxidising
Sodium chloride, NaCl	--
Sodium hydrogen carbonate, NaHCO <sub>3</sub>	--
Sodium nitrite, NaNO <sub>2</sub>	toxic
Sulfanilamide, C <sub>6</sub> H <sub>8</sub> N <sub>2</sub> O <sub>2</sub> S	--
Low-nutrient seawater: See Note 2.	

### REAGENT MAKE-UP

DI Water refers to high quality distilled or deionized reagent water, Type I or Type II as defined in ASTM Standards, Part 31, D 1193-74.

### 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 1 liter with DI water.

### SYSTEM WASH SOLUTION

Brij-35, 30% solution	6 mL
DI water	to 1000 mL

Add 6 mL of Brij-35 to 1000 mL of DI water.

### AMMONIUM CHLORIDE REAGENT

Ammonium chloride	10 g
Ammonia solution, 25%	pH adjustment
DI water,	to 1000 mL
Brij-35, 30% solution	0.5 mL

Dissolve 10 g of ammonium chloride in about 900 mL of DI water and adjust the pH to 8.5 ± 0.1 with ammonia solution (25%). Dilute to 1000 mL with DI water. Add 0.5 mL of Brij-35 per liter and mix thoroughly. Replace weekly. See operating note 10.

**COLOUR REAGENT**

Sulfanilamide	10 g
Phosphoric acid, conc.	100 mL
N-1-Naphthylethylenediamine dihydrochloride	0.5 g
DI water	to 1000 mL

To approximately 700 mL of DI water add 100 mL concentrated phosphoric acid and 10 g of sulfanilamide. Dissolve completely. Heat if necessary. Add 0.5 g of N-1-naphthylethylenediamine dihydrochloride and dissolve. Dilute to 1000 mL with DI water and mix thoroughly. The reagent should be colorless: if it is pink the phosphoric acid is probably impure. Store in a cold, dark place. Stability: one month.

**STOCK COPPER SULPHATE SOLUTION, 2%**

(Used to prepare cadmium column)

Copper sulphate	2 g
DI Water	to 100 mL

Dissolve 2 g of copper sulphate in about 60 mL of DI water. Dilute to 100 mL with DI water and mix thoroughly. Stability indefinitely.

**6N HYDROCHLORIC ACID**

(for cadmium column preparation)

Hydrochloric acid	495 mL
DI Water	to 1000 mL

Cautiously, with swirling, slowly add 495 mL of hydrochloric acid to about 400 mL of DI water. Cool down to room temperature. Dilute to 1000 mL with DI water and mix thoroughly. Stability indefinitely.

**STANDARDS**

(See operating note 2)

**STOCK STANDARD NITRATE, 100 mg N/L**

Potassium Nitrate	0.722 g
DI Water	to 1000 mL

Dissolve 0.722 g of potassium nitrate in DI water. Dilute to 1000 mL and mix thoroughly. Store in a dark bottle.

**WORKING STANDARDS NITRATE**

Prepare working standards as required.

**STOCK STANDARD NITRITE, 100 mg N/L**

Sodium Nitrite	0.493 g
DI Water,	to 1000 mL

Dissolve 0.493 g of sodium nitrite in DI water. Dilute to 1000 mL and mix thoroughly. Store in a dark bottle.

**WORKING STANDARDS NITRITE**

Prepare working standards as required.

## OPERATING NOTES

1. For seawater analysis use a sampler wash solution containing sodium chloride and sodium hydrogen carbonate if the method will be run at 60 samples per hour. For water and wastewater analysis use only DI water. It is possible to use DI sampler wash solution for seawater analysis, but the sampling rate will need to be reduced because there will be a disturbance at the beginning and end of each peak. The peak window on the AACE Software is then programmed to ignore the disturbance.
2. For the most accurate results, the standard diluent should have the same matrix as the samples. Therefore, use artificial seawater or low-nutrient seawater for seawater analysis. To avoid errors from silicate 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.

### 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 1 and 2).
- § The prepared reagents should be degassed by vacuum membrane filtration for best performance. Filter with a pore size of 0.5  $\mu\text{m}$  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 13. 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 15).

4. The nitrite concentration can be determined by eliminating the reductor column and standardizing with an appropriate nitrite solution. In order to determine the nitrate values, the nitrite alone must be subtracted from the total (nitrate and nitrite). Check the efficiency of the reductor column regularly by analysing equi-molar nitrate and nitrite standards. The reduction efficiency should be > 95%. See also operating note 5(k).

## 5. Reductor Column

- a Cadmium of a granulation of 0.3 to 0.8 mm should be used. See operating note 4(i).  
Danger: cadmium is toxic. Avoid skin contact or breathing the dust.
- b New or used cadmium particles are cleaned with 50 mL of 6N HCl for one minute. Decant the HCl and wash the cadmium with another 50 mL of 6N HCl for one minute. About 10 g of cadmium is needed for 1 column; however, for convenience a larger quantity can be prepared at once, either up to the end of step (g) or as complete packed columns filled with ammonium chloride solution and sealed.
- c Decant the HCl and wash the cadmium several times with distilled water.
- d Decant the distilled water and add 50 mL of 2% copper sulfate pentahydrate per 20 g cadmium. Wash the cadmium until no blue colour remains in solution. If using less cadmium, use proportionately less copper sulfate.
- e Rinse the cadmium several times with distilled water then decant.
- f Add another 50 mL (or suitable volume) of 2% copper sulfate pentahydrate and wash until no blue colour remains in solution.
- g Decant and wash thoroughly with DI water until the washings contain no more black colloidal copper. The cadmium granules should look silvery. If the cadmium is not washed completely, small particles of copper will escape and produce noise. IMPORTANT: from this point on, do not allow the cadmium to come into contact with air. Always keep it covered with water or  $\text{NH}_4\text{Cl}$  reagent.
- h Close one end of the reductor column with glass wool. Fill the reductor column with ammonium chloride reagent and transfer the prepared cadmium particles to the column using a syringe. Be careful not to allow any air bubbles to be trapped in the column. Close the filling side with glass wool.
- i As an alternative to the glass Reductor Tube, if using Cd larger than 0.8 mm, use 12 - 18 cm of Tygon tubing 116-0536-18 (see operating note 5k). Fill the Tygon tubing with ammonium chloride reagent and transfer the prepared cadmium particles to the column using a Pasteur pipette or a small funnel or syringe. Be careful not to allow any air bubbles to be trapped in the column.
- j Prior to sample analysis, condition the column by pumping through the sample line a 100 mg/L (nitrate) standard for five minutes followed by 100 mg /L (nitrite) for ten minutes. When the 100 mg/L standard is washed out completely, pump the top standard through the column and continue until the response is stable (it could takes about 30 minutes). This procedure stabilises the performance of the column.
- k The required column dimensions can be adjusted by using different tubing, depending on the cadmium grain size and porosity. The lowest dispersion is obtained when the column diameter is twice the size of the largest granule. The length may be adjusted to ensure complete reduction and an acceptable time between regeneration. Excessive length or dead volume will cause higher carryover.
- l When the reduction efficiency falls to an unacceptable level, empty the column and repack it with regenerated cadmium. Old cadmium can be kept and re-used. If cadmium is discarded, be sure to comply with local regulations for disposal of toxic metals.

**6. Start-up Procedure**

- a Check the level of all reagents to ensure an adequate supply.
- b Place all lines in their containers and start the pump.
- c When the ammonium chloride reagent has reached the end of the C3 fitting, connect the reductor column, being careful not to get any air bubbles into the column.
- d Allow the system to equilibrate.

**7. Shut-down Procedure**

- a Stop the pump and remove the column. Block off the ends of the column to eliminate any loss of the ammonium chloride reagent. Connect the C3 fitting to the injection fitting (116-0489-01) with a piece of 0.034" I.D. polyethylene tubing.
- b Start the pump, remove all reagent lines and place them in distilled water containing 1 mL/L Brij-35.
- c After ten minutes stop the pump and remove the platen.

**8. Reagent Background Colour**

- a Pump distilled water containing 1 mL/L of Brij-35 through the system.  
NOTE: The cadmium column should not be connected at this time.
- b After making all necessary adjustments on the Colorimeter, set the Std. Cal. control to 1.00 (this sets full-scale absorbance to 1.0), and with the Baseline control, adjust the water baseline to zero.
- c Follow the start-up procedure described in operating note 6.
- d The reading of the reagents compared to distilled water should not be more than 0.04 absorbance (4%). If the absorbance is greater than six units, one or more of the reagents or the water used to make up the reagents is probably contaminated.

9. To extend the life of the column between regeneration use nitrogen instead of air for the segmenting bubbles.

**10. Brij-35**

Old or oxidised Brij-35 can cause poor peak shape and low recovery. Do not use ready-prepared 30% solution after the expiry date, and keep the bottle closed.

**10. Additional performance data:**

	<b>Sample A</b>	<b>Sample B</b>
Lag time	6 min.	7 min.
Carryover	0.3%	

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

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

13. Manifold cleaning procedure:

Every day use system wash solution. Once a month, wash the manifold, including the sample line, with diluted hypochlorite solution containing a little Brij, after disconnecting the Cd column. Then rinse completely with system wash solution.

14. For total N, prepare a digestion reagent according to the procedure of K. Grasshoff et. al. (Methods of Seawater Analysis, 2<sup>nd</sup> Edition, Verlag Chemie, 1983) as follows:

15g H<sub>3</sub>BO<sub>3</sub>, 25g K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, 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 115°C for 2 hours. The pH of the digested samples should be about 8.

15. 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 (the 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 (17).

16. 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.

17. 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, July 2000

Added nitrite standard, small text revisions, added Total N digestion procedure.

### Revision 3, March 2001

Added operating note 18.

### Revision 4, March 2001

Added glassware inside AA3 colorimeter, corrected operating notes 5 and 6.

### Revision 5, March 2001

Added 4 way valve to by-pass Cd column.

### Revision 6, August 2001

Added range and performance data for AA3 system to first page, removed AAI data and notes, decreased max. Cd particle size to 0.8 mm.

### Revision 7, February 2002

Added LED photometer, expanded notes

### Revision 8, May 2002

Correction of the flowchart.

### Revision 9, December 2002

Changed pump tube air to blk/blk (0.32).

### Revision 10, March 2005

Added Note 17.

### Revision 11, May 2008

Changed logo

### Revision 12, July 2009

Air supply and de/rebubbling optimized, flowchart and consumables list updated.

**CONSUMABLES**

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

<u>Description</u>	<u>Legend</u>	<u>Part Number</u>	<u>Est. Annual Usage</u>
ORN/GRN, 0.10 mL/min		116-0549-04	1 pkg./12
ORN/WHT, 0.23 mL/min		116-0549-06	2 pkg./12
BLK/BLK, 0.32 mL/min		116-0549-07	2 pkg./12
ORN/ORN, 0.42 mL/min		116-0548-08	1 pkg./12
WHT/WHT, 0.60 mL/min		116-0549-09	1 pkg./12
YEL/YEL, 1.20 mL/min		116-0549-12	1 pkg./12
PUR/WHT, 3.90 mL/min		116-0549-18	1 pkg./12
Air valve tubing, Pharmed		117+0539-07	1 m
Polyethylene tubing 0.015" ID	PE15	562-2002-01	1 m
Polyethylene tubing 0.03" ID		562-2015-01	1 m
Tygon tubing	T07	116-0536-07	2 m
Tygon tubing		116-0536-11	1 m
Tygon tubing		116-0536-16	1 m
Tygon tubing (for nitrate)		116-0536-18	1 m
Kel-F tubing 0.050" x 0.08"	KF14	562-3014-01	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+B045-10	1 pc.
AA3 Filter Assy, 550 nm		165+B044-55	1 pc.
AA3 Colorimeter lamp		169+B143-01	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, 5 turns right	5TR	170-0103-01	1 pc.
Glass coil, 5 turns left	5TL	170-0426-01	2 pcs.
Glass coil, 10 turns right	10TR	157-0251-01	1 pc.
Glass coil, 10 turns left	10TL	157-0226-01	1 pc.
Glass tubing	p	170-0187-01	1 pc.
Glass tubing	r	170-0193-01	1 pc.
Glass tubing U	ae	116-0223-48	1 pc.
Connector T	A10	116-B034-01	4 pcs.
Connector T	C3	116-0202-03	1 pc.
AA3 Heater assembly		169+B410-01	1 pc.
AA3 Coil, 5.37 mL		169+B442-01	1 pc.
AA3 Controller, 115V/230V		169+B430-01/02	1 pc.
Thermometer 32-42°C		157-0283-01	1 pc.
4 way valve (for nitrate)	4WV	566+0010-01	1 pc.
Nut		508+2011-01	2 pcs.
Nut		508-2011-06	2 pcs.
Fitting		508+2012-01	4 pcs.

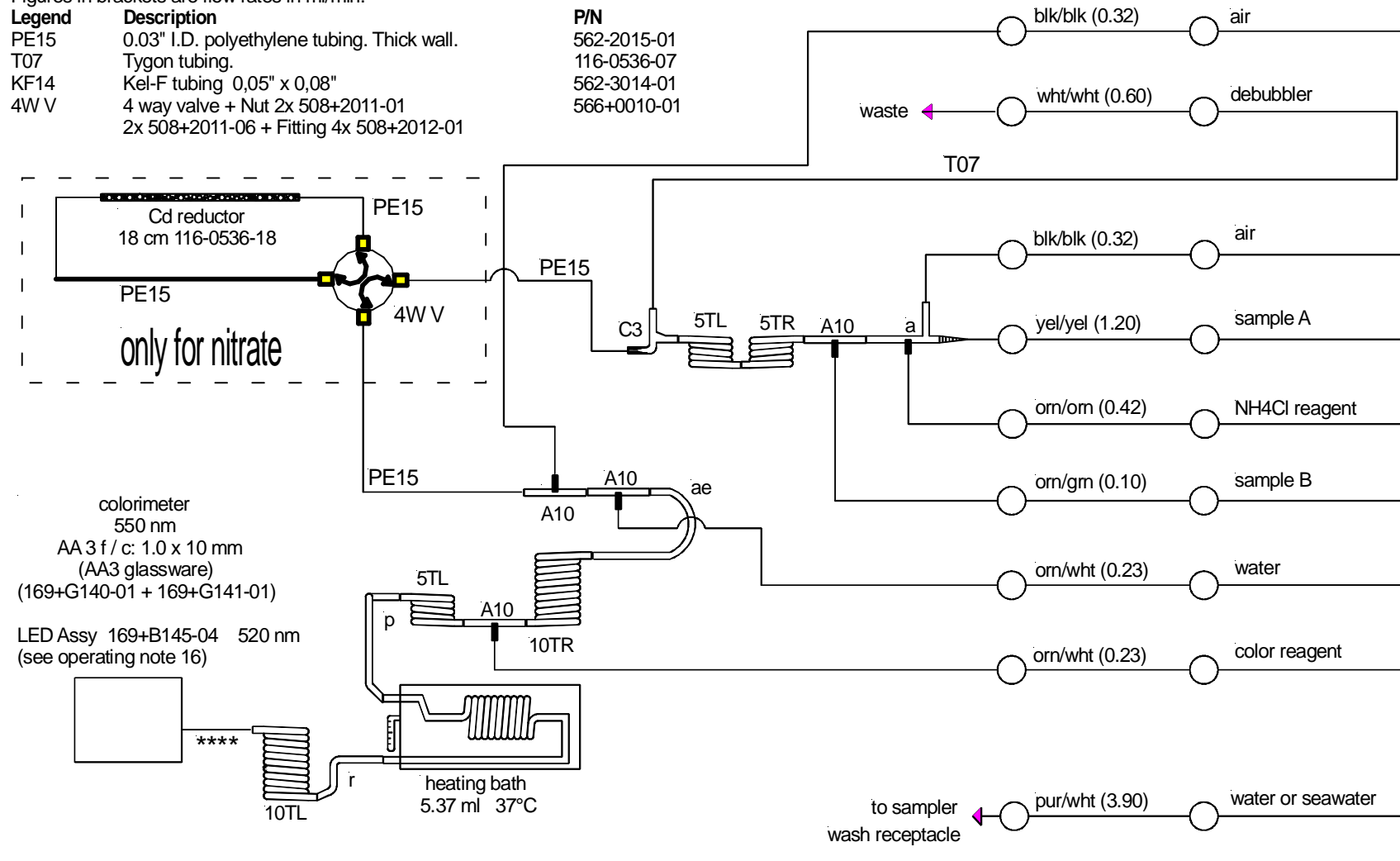


**NOTES :**

Figures in brackets are flow rates in ml/min.

Legend	Description
PE15	0.03" I.D. polyethylene tubing. Thick wall.
T07	Tygon tubing.
KF14	Kel-F tubing 0,05" x 0,08"
4W V	4 way valve + Nut 2x 508+2011-01 2x 508+2011-06 + Fitting 4x 508+2012-01

P/N
562-2015-01
116-0536-07
562-3014-01
566+0010-01



DRAWN	S.Giedigkeit	01.03.99	SYSTEM	AA3	PARAMETER	Nitrate / Nitrite
CHANGED	S.Giedigkeit	07.07.2009	METHOD NO. (11)	G-172-96	MATRIX	Water and Seawater
RELEASED	U. Grummisch	07.07.2009	REMARK	Multitest MT19	RANGE	Sample A: 0-2.9 to 0-46 µmol/l as N Sample B: 0-50 to 0-500 µmol/l as N



**PROPRIETARY NOTE**  
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