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Measuring ammonium (NH_4^+) concentrations in water samples

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Fluorometric assay for precise measurements of ammonium from 0.3 to 50 µmol•L-1 concentrations. Reaction reaches maximum fluorescence after ~2 hours and remains stable for several more hours. Single, non-hazardous working reagent is stable for months. NOTE: This protocol is written for measurement in 96-well plates—adjust reaction volumes if using fluorometer for measurements.
Reference: Holmes, R.M., A. Aminot, R. Ke?rouel, B.A. Hooker, B.J. Peterson. (1999) A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Can. J. Fish. Aquat. Sci. 56: 1801–1808.

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Fluorometer (or plate reader)
200 and 1000 µL filter tips
200 and 1000 µL pipettes
microcentrifuge tubes
Tube racks
15 mL conical tubes (for standards)
Vortexer
96-well microplate with lid, black (preferable)
Working reagent
Ammonium standards

1 1. Prepare 200 µM stock solution: Dilute 1:500 from 0.1 M solution => 20 µL + 9.980 mL nanopure water.

2 Dilute the stock solution to the following concentrations (µM) in nanopure water: 0, 0.5, 1.25, 2.5, 5, 7.5, 10. NOTE: 1 mL of each standard is necessary for triplicate reactions.

3 Prepare Reagents and Solutions.

3.1 1. Sodium sulfite solution: Add 1 g of sodium sulfite (Sigma S-4672) to 125 mL of nanopure water. The resulting solution is stable for ~1 month when stored at room temperature in a glass bottle.

3.2 2. Borate buffer solution: Add 80 g of sodium tetraborate (Sigma S-9640) to 2 L of nanopure water. Stir or shake thoroughly to dissolve.

3.3 3. OPA solution: Add 4 g of OPA (Sigma P-1378) to 100 mL of ethanol (use a high-grade ethanol because impurities in ethanol can autofluoresce). OPA is light sensitive, so it should be protected from light while dissolving in ethanol and stored in the dark.

3.4 4. Working Reagent: In a large (>2 L) brown polyethylene bottle, mix 2 L of borate buffer solution, 10 mL of sodium sulfite solution, and 100 mL of OPA solution. Ideally, allow the WR to “age” for 1 day or more prior to use because its blank will decrease over time. The resulting WR is stable for at least 3 months when stored in the dark at room temperature, or longer when stored at 4ºC. The final WR should contain the chemicals at the following final

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concentrations: borate buffer (40 g·L−1, 21 mM), sodium sulfite (40 mg·L−1, 0.063 mM), and OPA in ethanol (50 mL·L−1).

4 A. Assay set-up.NOTE: This procedure can be modified for 24-well plates by increasing reaction volumes 4X and preparing reactions in 15 mL conical tubes. 24-well plates hold up to 2.5 mL per well.

4.1 1. Label one microcentrifuge tube for each sample, blank, and all standards (7).

4.2 2. Working under dimmed light, aliquot 1 mL of Working Reagent (or nanopure water, as appropriate) to each reaction (or blank) tube.

4.3 3. Add 250 µL of sample, standard, or nanopure water to each corresponding tube.

4.4 4. Mix (invert or vortex) and incubate in the dark at room temperature for 2-3 hours.

4.5 5. Transfer 250 µL of each reaction to triplicate wells of a 96-well microplate and measure fluorescence on plate reader.

5 B. Reading plates.

5.1 1. Turn on Tecan Infinite 200 PRO plate reader 20-30 minutes prior to use.

5.2 2. Once warmed up, open the iControl software on MLCLab-PC.

5.3 3. Open file “OPAammonia_96well” (or “OPAammonia_24well” if appropriate).

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5.4 4. Load the plate—check whether the “plate with cover” box is checked (can read with lid if using clear plates but make sure to remove lid if using black plates).

5.5 5. Read plate at 350±9 nm excitation and 422±20 nm emission (fluorescence top mode, manual Z-position set to 24995 µm, 25 flashes, manual gain of 100, 20 µs integration time). Program automatically opens an Excel file that documents parameters and data.

6 C. Analyzing data.

6.1 1. Subtract the fluorescence values of the samples mixed with nanopure water (or borate buffer, preferably) instead of the Working Reagent (sample blanks) from the corresponding reacted sample fluorescence’s (= corrected sample fluorescence).

6.2 2. Subtract the average fluorescence of the nanopure water tubes (i.e., 0 µM ammonium) mixed with borate buffer instead of Working Reagent (standard blanks) from the fluorescence values of all the standards.

6.3 3. Plot corrected fluorescence (y) vs. concentration (x) for all standards to establish a standard curve with linear regression.

6.4 4. Use the equation of the standard curve to calculate sample concentration from fluorescence.

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