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15N2 label preparation (dissolved method)

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

A protocol for making pre-dissolved 15N2 label in sterile seawater

PROTOCOL CITATION

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MATERIALS TEXT

Side arm flask
Vacuum pump (900mbar)
Vacuum tubing
Magnetic stir block
Magnetic stir rod
4x 500mL serum bottles (Wheaton 223952)
30mm rubber septa (Wheaton W224100-173)
30mm crimp sealer
30mm crimp seals (Wheaton 224187-01)
Needles
20mL syringes
$^{15}N_2$ gas (Cambrige isotopes)
0.22um filters
Vacuum filtration unit or peristalic pump with filter heads

Calculations

1 The following protocol is based on the dissolved gas method described by Mohr et al. 2010 and Klawoon et al. 2015.

This protocol makes 4x 500mL serum bottles of $^{15}N_2$ labels enriched to ~90%. Enrichment of each serum bottle depends on the vacuum pressure used for degassing. The following calculations can be used to estimate degassing potential based on the vacuum pressure.

$[N2]_{seawater}$ = 0.000398 mol/L = 398 mol/L at 24°C, 1 atm

$[N_2] = TGP * X_{N2} * k_H$

$[N_2]$ is the concentration of N_2 after reaching equilibrium at pressure *p.*

TGP is the total gas pressure, which is the difference between atmospheric pressure (p_{atm}) and vacuum pressure (p_v). (TGP = p_{atm} - p_v).

X_{N2} is the mole fraction of dinitrogen gas. X_{N2} = 0.7804

k_H is Henry's Law constant for nitrogen gas at 35ppt and 24°C. k_{H} = 0.00051 mol/L atm

A	B	C	D
pv (mbar)	pv (atm)	[N2] mol/L	Degassing %
200	0.20	0.000319444	20
600	0.59	0.000162324	59
950	0.94	0.000024845	94

Amount of disssolved N_2 that can be theoretically degassed after equilibrium is reached at the following vacuum pressures, assuming a temperature of 24°C and salinity of 35.

References:

Klawonn, I., Lavik, G., Böning, P., Marchant, H., Dekaezemacker, J., Mohr, W., & Ploug, H. (2015). Simple approach for the preparation of 15− 15N2-enriched water for nitrogen fixation assessments: evaluation, application and recommendations. *Frontiers in microbiology*, *6*, 769.

Mohr, W., Grosskopf, T., Wallace, D. W., & LaRoche, J. (2010). Methodological underestimation of oceanic nitrogen fixation rates. *PloS one*, *5*(9), e12583.

Preparation

2 Wash all glassware, tubing, rubber septa, etc. with 10% HCl and then rise 4x with Elga water (18.2MΩ).

3 Filter sterilize 3L of seawater using 0.22um pore size filters. Only ~2.5L of this is needed to fill serum bottles but it is good to have some extra when siphoning (step 5).

Make label

4 Using a vacuum flask, vacuum pump, magnetic stir plate and stir rod, degas 2L of water at ≥ 900 mbar for at least 20 minutes.

5 Using acid washed tubing, transfer degassed water from the vacuum flask to serum bottles by creating a siphon. Use a syringe to start the siphon.

Using a siphon avoids pouring the water an potentially reintroducing gas into the water. Take care to keep the tubing ends at the bottom of both the flask and the serum bottle to avoid splashing.

6 Using the siphon, fill serum bottles headspace free and crimp seal.

Depending on the rubber septa used, this step can be tricky and requires a bit of practice to not introduce an air bubble when inserting the septa.

7 Using a needle and syringe, inject $^{15}N_2$ gas in a ratio of 2ml/100mL into serum bottles (i.e. 11 mL for a 500mL serum bottle, as the headspace free volume is 550mL). A second needle and syringe should be used during injection as an outflow.

7.1 When preparing gas syringe, flush any tubing/syringe being used 2x with $^{15}N_2$ gas to remove any air.

7.2 Turn the serum bottle upside-down. Insert both the input and output needles/syringes into the septa. Slowly inject the gas label. Injecting upside prevents any gas from escaping through the outflow.

7.3 After all the gas is injected, water accumulated in the outflow can be used to re-pressurize the serum bottle. Re-pressuring can increase dissolution (see Klawonn 2015 et al. Front. Microbiology)

If there is any air in the outflow syringe, purge this air out prior to re-pressurizing so that only degassed water is being used to re-pressurize the bottle.

8 Shake serum bottles for a few minutes and then store horizontally, such that the gas bubble is not in contact with the septa. Store for 24 hours prior to use to increase dissolution (see Klawonn 2015 et al. Front. Microbiology)

Note: Injecting 100mL of this water into 1L incubation chambers gives an enrichment of ~9.7 atom% 15N2

Illustration of protocol

9 degassing_illustration.png

Illustration by M. Moynihan