

DOC Experiment Protocol

Written 03/05; last updated 09/08 WKoz

EQUIPMENT

Standard RAD filtration set-up (will only need four out of the six funnels)
4-Place DOC filtration rack with Erlenmeyer flasks (in wooden box)
2 vacuum pumps (1 with standard set-up, 1 additional for DOC manifold)
3.0 μm filters
0.22 μm filters
P5000 Pipette
P200 Pipette
P100 Pipette
500 μl Eppendorf Repeat Pipettor (set to "2" for 200 μl , with a 5ml tip)
1, 20 ml scintillation vial
2, 100 ml graduated cylinders (one hot, one not)
28 incubation bottles in wooden box (14 light, 14 dark) + 2 additional bottles for t_0
188 numbered 7ml scintillation vials
8 vial racks
Flow-through incubation bath with 25% (2 grey) or 10% (2 grey, 1 black) light screening (depends on ambient light and procedure at the time)
1M NaOH
20% HCl
Nanopure Water
Fresh 0.22 μ Filtered Sea Water
 ^{14}C stock

SAMPLE WATER

~ 2L from 50% light depth

^{14}C STOCK DILUTION

260 μl ^{14}C (assuming 2mCi/ml stock concentration) with 3.0 ml Nanopure water in 20ml scintillation vial

SETUP

For time zero (t_0) samples, only load the first two places in the standard RAD filtration rack with 3.0 μm filters, and two 0.22 μm filters into the first two places in the DOC funnels.

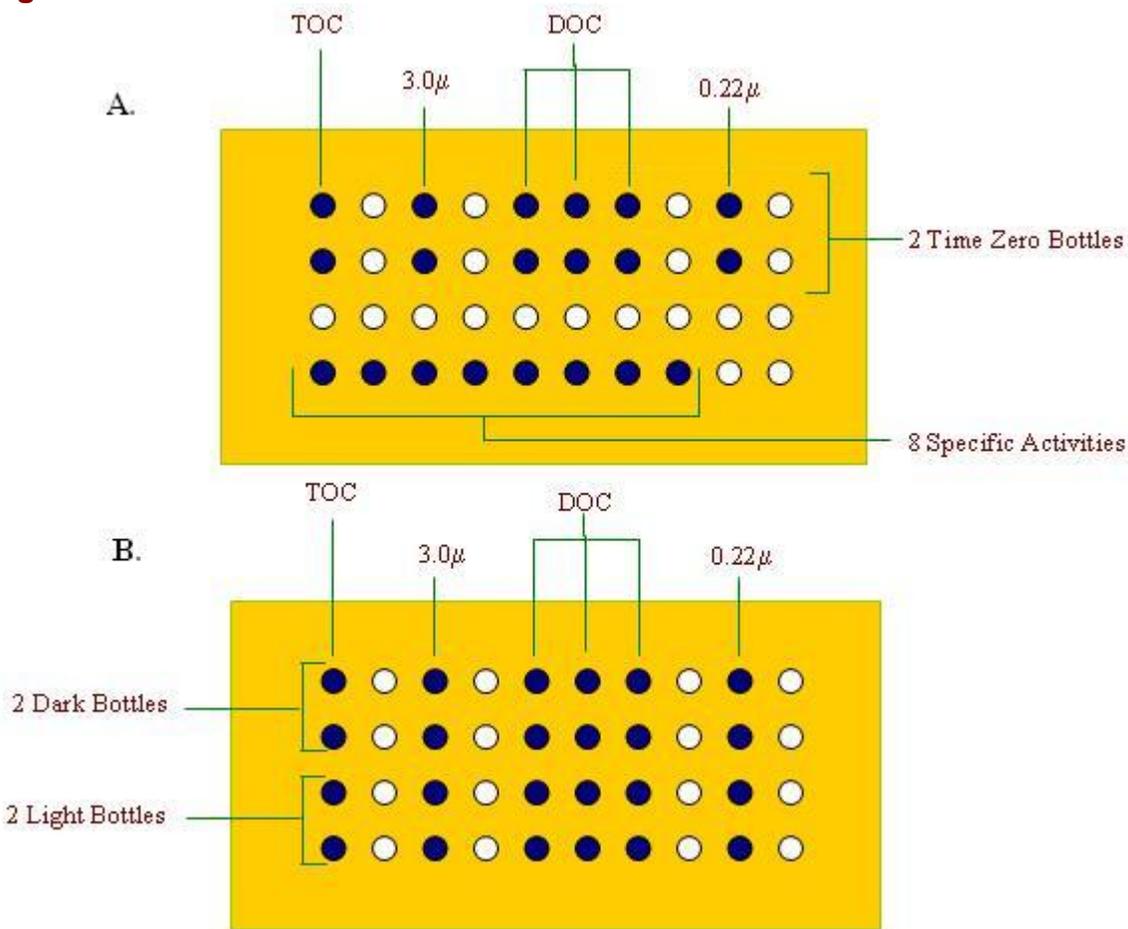
For all remaining time periods, load all 4 funnels in each rack with appropriate filters.

**Hint: The 3.0 μm filters are fairly brittle, so after you've tightened the Gelman cup onto the filter base, check that there are no tears on the filter. This will save a lot of*

heartache later if you catch it before adding the water!

Lay out vials for each time step. Vials for specific activity and time zero can be placed together in the same rack – 8 SA vials and 12 t_0 vials (Figure 1a). The other time steps fit best in separate racks – 24 vials (4 sets of 6 vials) for each time step (Figure 1b).

Figure 1



PROCEDURE

Measure 50ml of sample water into each of the 30 incubation bottles (14 light, 14 dark, 2 light time zeros).

Pipette 100μl of diluted ^{14}C into each incubation bottle. Cap and mix bottles.

Prepare 8 Specific Activity Vials:

Specific activities are taken from any three light bottles, any three dark bottles, and each of the time zero bottles.

For each specific activity, pipette 100μl NaOH and 100μl of the spiked sample water.

Add scintillation cocktail immediately.

Place the 14 light and 14 dark bottles into incubation bath on the back deck, and cover with 25% or 10% light screening (see equipment for screening).

Record start time for incubation.

Process time-zero bottles:

For each bottle, pour 20ml of sample water into filter funnel with 3.0µm filter.

Pour another 20ml into funnel with 0.22µm filter.

Before turning the pump on, pipette a 2ml sample from the bottle and place it into the TOC scintillation vials (total organic carbon).

Check that the pump pressure is set to 5 in. Hg. You'll probably have to decrease the pressure on the pump used for SIS experiments.

After filtering water, place the 3.0µm and 0.22µm filters into their own scintillation vials (POC samples).

From the filtrate of the 0.22µm filter, pipette three 2ml sample and place into each of three scintillation vials for DOC (dissolved organic carbon).

Acidify all samples immediately with 0.2ml of 20% HCL.

Every Hour for six (6) Hours:

Remove 2 light and 2 dark bottles from the incubation bath. Process each bottle in the same manner as the time zero bottles:

2ml TOC samples, 3.0µm filter, 2ml DOC samples in triplicate, and 0.22µm filter.

After eight (8) Hours:

Sample last 2 light and 2 dark bottles as per explanation above

After 12-24 hours:

Add 5ml scintillation cocktail to each vial. Cap and shake. Read DPMs in the Liquid Scintillation Counter.

Vials with 2 ml of seawater need to be thoroughly mixed. Use a vortex if needed.

Data analysis:

Enter DPMs in spreadsheet (phyto-bacteria/DOCyear-#) If data is noisy, in particular the DOC samples, re-acidify, mix all vials again in vortex or by hand and recount all experiment, starting with the last sample first.