Bacterial Productivity Protocol Using Thymidine Uptake for Fresh and Salt Water Samples
Florida Coastal Everglades LTER

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*Radioactive isotope use requires successful completion of Radiation safety training via F.I.U. Department of Environmental Health and Safety. In case of emergency of isotope spillage contact Radiation safety officer Dr. Dua (305) 348-2548.

Equipment:

- Automatic liquid scintillation counter
- Vaccum filtration manifold

Supplies:

- Record keeping book
- 50 ml centrifuge tubes and racks
- 20 ml glass scintillation vials and carrying tray
- 100 µl, 1000 µl and 5 ml adjustable pipettes
- Autoclaved 1000 µl pipette tips
- Lab coat, safety goggles and gloves
- Forceps (two, one for fresh filters and one for removing the radioactive filter)
- Three 40 ml autoclaved beakers and ice bath containers
- Nitrocellulose filters
- Ice cooler (s)
- Gaskets and stoppers to be used with the filtration manifold.
- ^{3}H waste receptacle

Chemicals:

- ^{3}H Thymidine stock solution
- ^{3}H Thymidine working solution (100 µl per blank and sample replicate)
- 50% TCA (1 ml per blank and sample replicate)
- 5% TCA (3 ml per blank and sample replicate)
- 80% Ethanol (non-denatured) (3 ml per blank and sample replicate)
- Phosphate buffered saline (PBS) solution:
  5 mM $NaH_2PO_4.H_2O$ (M.W.137.99)
  5 mM $Na_2HPO_4.7H_2O$ (M.W. 268.07)

130 mM NaCl (M.W. 58.44)

1. For 1L dissolve 0.69 g $NaH_2PO_4.H_2O$, 1.34 g $Na_2HPO_4.7H_2O$ and 7.6 g NaCl in 800 ml of distilled water.
2. Adjust pH to 7.5 with 1N HCl or 1N NaOH (1N 40g NaOH - 1 L)
3. Bring to 1 L with distilled water.
4. Autoclave at 121°C for 15 minutes.
5. Store PBS in the refrigerator between uses.

- 20% PBS buffered formaldehyde

1. For 1 L add 540.5 ml of 37% formaldehyde to 459.5 ml PBS buffer. Store this solution in a sterile 1000 ml container.
2. Filter sterilized the 20% PBS formaldehyde solution through a 0.2 μm filter into a 50 ml centrifuge tubes.

- ^3H Thymidine stock solution (example)
  Isotope Batch 207 (2/01)
  Specific Activity = 86.0 Ci/mmol (86.0 mCi/μmol)
  Radioactive concentration = 1.0 mCi/ml
  Yields quantity: 5 mCi

NOTE:
1. Keep ^3H – Thymidine at 2ºC.
2. Record Specific activity for each new batch and run for theoretical v. actual check.

- ^3H Thymidine working solution (example for specific activity = 86.0 Ci/mmol)

Stock Thymidine concentration:
(10 mCi/10 ml) x (1 μmol/86.0 mCi) = 0.0116 μmol/ml
(0.0116 μmol/ml) x (1000 ml/l) = 11.63 μM

-need 10 nM final concentration ion (15 samples/month) x 3 replicates + 1 blank = 60 samples

-assume we add 100 μl of working solution to each of the 60 samples = 6 ml.

x of 11.63 μM = 10 ml of 0.01 μM, where 10 ml is sample volume

x = 0.0086 ml

60 x 0.0086 ml = 0.516 ml Stock/month
Calculation check:

0.516 ml of 11.63 μM = 6 ml of x = 1.00018 μM

0.1 ml of 1.0 μM = 10 ml of 0.01 μM

therefore
working solution = 0.516 ml of Stock in 6 ml of filtered sterile seawater (FSSW).

In a 20 ml glass scintillation vial add 0.516 ml of isotope to 5.484 ml sterile SW using a sterile pipette tip and aseptic technique and mix. Store vial with diluted isotope at 2ºC in a labeled receptacle in VH-321 refrigerator station G.

1. Sample collection

1. Water samples are collected in clean, sample-rinsed dark polypropylene bottles filled by hand.
2. Samples are kept on ice during the sampling day.
3. Samples are stored at 4ºC in the laboratory and processed the next day.

2. Sample Incubation and ^{3}H uptake Experiment

1. Prepare three replicate 10 ml samples for each sample collected and pipette into separate centrifuge tubes.
2. Include one replicate of water from each sample from the batch to use as blanks with a final concentration of 2% bacteria free Formalin using PBS.
3. Add 100 μl of the ^{3}H working solution to each of the samples including the blanks.
4. Let samples incubate 1 hour in hood.
5. Add 1 ml of 50% TCA (50.0 g TCA in 100 ml of sterile, distilled water) to give a final concentration of 5% TCA.
6. Place the sample on ice or in freezer that is labeled for radiation. Samples should remain on ice for 15 to 60 minutes but no longer than 1 hour.
7. Prepare 2 beakers with ice cold 5% TCA and 80% Ethanol (non-denatured).
8. Place nitrocellulose filters (0.22 μm) shiny side-up on filtration manifold and ensure a tight seal of the tower cover.
9. Gently shaking the sample, decant the sample into the filtration tower and filter at low vacuum until filter is dry.
10. Rinse the tower three times with 1 ml portions of ice cold 5% TCA.
11. Rinse the filter three times with ice cold 80% ethanol.
12. Remove filter from manifold and place in 20 ml scintillation vial.
13. Clean up work areas and lab ware and conduct wipe test of each station A-I (see Isotope Records book VH-321 for map).
14. After 30 minutes add 10 ml scintillation cocktail (using the repeat decanter) to each scintillation vial with filter.
15. Let samples sit overnight and run on scintillation counter the next day.

3. Scintillation Counter Analysis:

1. Place scintillation vials in racks for liquid scintillation counter and apply tags to racks (user number and rack number + halt rack and tag).
2. Place racks with samples into the machine making certain the groove of the rack is aligned with the chain guided track of the LSC sample conveyer.
3. Record user information in LSC log book.
4. Press user number and go to user # 1 and check that user # 1 is ^{3}H press 1 for user number and ID.
5. When run data and quench curve data appear press auto-count.

4. Bacterial Productivity Calculations and Conversion Factors.

moles thymidine L^{-1} h^{-1} = [(dpm_{sample} - dpm_{blank})* (4.5 x 10^{-13})/SA * t *v]*(10^{-3})*(1.03)

where 4.5 x 10^{-3} is the number of curies per dpm; SA is the specific activity of the [^{3}H]thymidine solution in curies per mmol; t is the incubation time in h; v is the filtered volume in l; 10^{-3} is mmol per mole; and 1.03 is a correction factor for volume of formaldehyde added (assuming 1% final volume; not needed if the entire incubated volume is filtered).

µg C L^{-1} h^{-1} = (moles 1^{-1} h^{-1}) * (cells $mole^{-1}$) * (Carbon $cell^{-1}$)

Thymidine conversion factor (TCF) = 2 x 10^{18} cells mol^{-1}
Carbon conversion factor (CCF) = 10 fg C $cell^{-1}$

µg C L^{-1} day^{-1} = µg C L^{-1} h^{-1} * 24

References:
Bell, Russel T. 1993. Estimating Production of Heterotrophic Bacterioplankton via Incorporation of Tritiated Thymidine In Handbook of Methods in Aquatic Microbial Ecology, Paul F. Kemp, Barry F. Sherr, Evelyn B. Sherr, Jonathan J. Cole (eds) Lewis Publishers, Boca Raton, Florida : 495-503.