Byron Crump
Horn Point Laboratory
University of Maryland Center for environmental Science
Cambridge, MD, 21613, USA

1July2005

Bacterial Production: ^{3}H-Leucine incorporation

Modified from:
Smith, D.C., and Azam, F., 1992, A simple economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-Leucine. Mar Microb Food Webs 6:107-114.
Jude K. Apple (Personal communication)
Stefan Bertillson (Personal communication)

Safety:
All handling of radioisotopes should be performed in a designated area with protection to avoid spills (tray, protective paper). All personnel should have completed radioactivity protection training. Use lab coat, latex gloves and eye protection. Dispose of radioactive waste properly.

Materials for incubations:
L-4,5-^{3}H-Leucine at 42.5 Ci/mmol
2 ml microcentrifuge tubes with screw caps and sealing O-rings.
Microcentrifuge racks
Microcentrifuge for centrifuging liquid off tube caps
1-10 μl pipette and tips for 5 μl isotope additions
100 μl pipette and tips for 90 μl trichloroacetic acid additions
10 ml pipette for water sample additions
1 bottle (~ 100 ml) 100% trichloroacetic acid (TCA)
Vortex
Timer
Radioactive Dry Waste container

Materials for processing:
Microcentrifuge for pelleting TCA-precipitated macromolecules
Aspiration rig (vacuum pump, side-arm flask, tubing, Pasteur pipette)
Microcentrifuge tube rack
5% TCA solution, ice-cold
Repeater pipette for aliquoting 1.5 ml of TCA
Liquid scintillation cocktail
Repeater pipette for aliquoting 1.5 ml of liquid scintillation cocktail
Vortex
Radioactive Dry Waste container
Radioactive Liquid Waste container

Leucine Stocks/Isotopes:
Use L-4,5-^{3}H-Leucine at 42.5 Ci/mmol. We want to add 5 μl of the working solution to

1.7 ml of water samples and thereby obtain a final concentration of 20 nM Leucine in incubated water samples. This concentration is usually above substrate saturation, but for highly productive systems it is wise (and recommended) to make a saturation curve. Throughout the steps, it is important to work aseptically with sterile solutions, needles, pipette tips, etc., and I suggest that you prepare several smaller aliquots of isotope working solution in sterile microcentrifuge tubes to avoid contamination of stocks. Freezing stocks could result in degradation of ^{3}H substrates (according to manufacturers), so even a minor contamination could lead to large experimental errors if experiments are performed over a longer time period.

Isotope useage:
1.445 μCi/tube or 5.78 μCi per experiment (4 tubes) at a final concentration of 0.85 μCi/ml.

Experimental methods:
- Mark microcentifuge tubes on the lids. Use at least triplicate vials and a blank for any water sample.
- Remove lids of the tubes (place them in front of the rack upside down)
- Add 5 μl isotope stock solution to the vials.
- Immediately add 90 μl 100% TCA to the blank vials.
- Pipette 1.7 ml water sample into each vial
- Cap vials. Mix tubes thoroughly
- Incubate for 30-60 minutes at *in situ* temperature.
- Stop the incubation by centrifuging liquid off tube caps (to prevent dripping), opening tubes, and adding 90 μl 100% TCA to the sample vials.
- Vortex vigorously
- Store in the refrigerator or on ice and return to the laboratory for processing.

Sample Processing:
- Place the tubes in Eppendorf 5417C Microcentrifuge (30 will fit at a time). Make sure centrifuge is balanced. Mark the outer edge of each tube with a sharpie to help locate the pellet.
- Centrifuge at 14,000 RPM for 10 min. Make sure that that the carousel is balanced and that the plastic carousel lid is firmly in place, otherwise centrifuge will not spin.
- Remove vials from centrifuge and uncap. Aspirate supernatant by slowly running the pipette down the middle of the tube. It is critical that you avoid sucking up the bacterial pellet. Although the pellet is often not visible, it forms about ¼ inch up the outside edge of the tube. Try to suck up every drop of liquid in the tube.
- Add 1.5ml of 5% ice-cold TCA to each tube and vortex well.
- Centrifuge at 14,000 RPM for 10 min. Make sure marked edges face out.
- Aspirate supernatant
- Add 1.5ml scintillation cocktail to each tube and vortex well.
- Place tubes in glass 20ml liquid scintillation vials, cap vials and count samples in liquid scintillation counter (for Packard Tri-Carb LSC in the AREL building, use protocol #2). The tubes do not have to be counted immediately.