Byron Crump **22 Feb 2020**

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.

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. When working in radioisotope vans on board ships always wear shoe covers and perform wipe tests daily.

Materials:
L-4,5-^{3}H-Leucine at ~42.5 Ci/mmol
2 ml microcentrifuge tubes with screw caps (Fisher Cat# 02-681-344 and 02-681-358)
Microcentrifuge racks
Picocentrifuge for spinning liquid off tube caps
1-10 microliter pipette and tips for 5 microliter isotope additions
100 microliter pipette and tips for 90 microliter trichloroacetic acid additions
1 microliter pipette for water sample additions
1 bottle (~ 100 ml) 100 % trichloroacetic acid
Vortex
Timer
Incubator
Microcentrifuge for pelleting TCA-precipitated macromolecules
Vacuum pump with liquid protection disk
Radioisotope aspiration system (Pasteur pipette, tubing, catch flask)
1 bottle (~1 L) 5% trichloroacetic acid (ice cold, prepared on ship from 100% stock)
Liquid scintillation cocktail
Repeater pipette for aliquoting 1.5 ml of liquid scintillation cocktail
Radioactive liquid waste container
Radioactive dry waste container

Leucine Stocks/Isotopes:
Use L-4,5-^{3}H-Leucine at 42.5 Ci/mmol. We want to add 5 ul 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 tritiated 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 uCi/tube or 5.78 uCi per experiment (4 tubes) at a final concentration of 0.85 uCi/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 ul isotope stock solution to the vials.
- Immediately add 90 ul 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 ul 100 % TCA to the sample vials.
- Vortex vigorously
- Store in the refrigerator or on ice and return to the laboratory for processing.

On-ship or laboratory processing:

- Mark the outer edge of each tube with a sharpie to help locate the pellet.
- Place tubes in microcentrifuge with mark facing out. Make sure centrifuge is balanced.
- Centrifuge at 14,000 RPM for 10 min.
- 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 precipitated macromolecule 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 1.5 mls 5% of cold TCA to each tube and vortex well.
- Place tubes in the centrifuge again, making sure marked edge is on the outside. Spin tubes at 14000 RPM for 10 min.
- Aspirate supernatant again.
- Add 1.5 ml scintillation cocktail to each tube and vortex well.
- Pour liquid from aspirator catch flask into liquid waste container.
- Store tubes in the dark at room temperature and wait at least two days for sample to combine with scintillation cocktail
- Place tubes in uncapped glass 20 ml liquid scintillation vials and count samples in scintillation counter.