Bacterial Production Microfuge Method

Updated to use 50% TCA (can replace 150 µL 50% with 80 µL of 100%)

1. Add appropriate labeled compound (³H-leucine or –thymidine) to microcentrifuge tube. 20nM is typically rate-saturating. Do 3 live and 1 killed control. Add 1.5 mL of sample.

2. Add 150 µL of 50% TCA to the killed control.

3. Incubate at in situ temperature in the dark for as little as 30min in the estuary, 1 hour in coastal waters and 2 hours in the Arctic basin.

4. Place on ice for 5 min. Most important for TdR, not leu.

5. Add 150 µL of 50% TCA (final conc 5%).

6. Centrifuge for 10min at high speed.

7. Pour out liquid (or pipette out, but avoid disturbing pellet at bottom).

8. Add 1mL of ice cold 5% TCA.

9. Invert tubes and repeat centrifuge step.

10. Pour out TCA.

11. Add 1mL of ice cold 80% ethanol.

12. Invert tubes and repeat centrifuge step.

13. Pour out EtOH.

14. Open tubes and allow to air-dry in hood.

15. Add 0.5mL of scintillation cocktail. Vortex. Allow to sit for 2 days before counting.

16. Vortex and radioassay in the scintillation counter.

Kirchman lab protocol

Questions? E-mail mattcott@udel.edu