

## Bacterial Production (Leu, TdR) Carlson Lab -- UCSB

### Preparation: Example (Specific activity and stock concentrations will vary)

	<sup>3</sup> H-TdR	<sup>3</sup> H-Leu
Specific Activity (μCi/nmol)	91	63
Stock [isotope] (μCi/ml)	1000	1000
H <sub>2</sub> O sample vol (ml)	1.6	1.6
Desired [isotope] (nM)	10	20
Calculation	$[(1.6)*(10)*(91)]/1000$	$[(1.6)*(20)*(63)]/1000$
Amount isotope to add (μl)	1.456	2.016

NOTE: Aliquot total amount isotope needed from stock vial into fresh microfuge tube, then dispense into individual tubes (avoids contamination).

### Protocol

1. Prepare triplicate tubes for every depth, plus one blank tube per depth. Also setup 3 tubes for specific activities.
2. Collect H<sub>2</sub>O samples in 30ml PC bottles (acid washed and dH<sub>2</sub>O rinsed).
3. In each sample tube place (in order):
  - i. Calculated stock of isotope (1.46μl TdR or 2.0μl Leu)
  - ii. 1.6ml H<sub>2</sub>O sample
4. In each blank tube place (in order):
  - i. Calculated stock of isotope (1.46μl TdR or 2.0μl Leu)
  - ii. 100μl 100% cold TCA
  - iii. 1.6ml H<sub>2</sub>O sample
5. Record the starting time of the incubation (since multiple samples are done, the starting time of the incubation is taken from the time sample is added to the first tube to the time sample is added to the last tube).
6. From 3 randomly selected samples take 10ul into a clean 2.0ml centrifuge tube for specific activities.
7. Incubate the samples in the dark and as close to *in situ* temperatures as possible. Record incubation temperatures.
8. After 2-3 hours (experiments have shown linearity of TdR incorporation to be up to 8 hours) end the incubation by adding 100ul 100%TCA, record the ending incubation time and temperature, and vortex the samples. **DO NOT add TCA to the specific activities.**
9. Store in the refrigerator until extraction. Can store tubes at 4°C for up to 48 hrs before extracting.
10. Extraction
  - i. Vortex samples if they have been sitting for a day or more. **Specific Activities are not extracted.**
  - ii. Spin 7min, 14000rpm, 4°C (mark tubes, lines out). *Aspirate to dryness, or decant.*
  - iii. Add 1.6ml 5% TCA
  - iv. Spin 7min, 14000rpm, 4°C. *Aspirate to dryness, or decant.*
  - v. Add 1.6ml 80% EtOH
  - vi. Spin 7min, 14000rpm, 4°C. *Aspirate to dryness, or decant.*

- vii. Add 1.5ml Ultima Gold. Vortex. Also, add 1.5ml Ultima Gold to Specific Activities and vortex.
- viii. Leave for at least 2 hrs, RTP (stable up to 1 month @ RTP)
- ix. Put into scintillation counter.