RUA # 1384

UNIVERSITY OF CALIFORNIA, DAVIS
ENVIRONMENTAL HEALTH & SAFETY, HEALTH PHYSICS

Radiation Use Authorization

Safety Protocol Addendum

Provide a copy of this protocol to each co-worker participating in the experiment.

Title of Project: Initiation of development in *Myxococcus xanthus*
Purpose To understand how changes in gene expression lead to changes in behavior and morphology.

Radioisotopes ^{3}H
Chemical Form: ^{3}H-Uridine and ^{3}H-Thymidine

A. What personal protection methods will be used to prevent contamination and internal exposures to radiation?
Disposable gloves __X__ Disposable booties_____ Lab coat __X__ Glove box_____
Fume hood __X__ (Flow rate: _____ FPM) Absorbent paper __X__ Respirator _____
Other: Lucite shields and boxes will be provide for storage and work shielding. All areas and equipment used for radioactive work will be clearly marked and shielded. A designated "hot bench" will be used for most radioactive work, this bench will contain a microcentrifuge, heating block, water bath and aspirator dedicated for radioactive work. No eating or drinking will be permitted near radioactive work areas.

B. How will you detect radioactive contamination and/or radiation fields?
Wipes and liquid scintillation counting

C. At what frequency will you check your laboratory for contamination?
After each experiment __X__ Biweekly __X__

D. Explain your method for decontamination of nondisposable objects contaminated with radioactivity:
Non-disposable items will be cleaned with "count-off" and water. Rinses will be placed in to the appropriate liquid radioactive waste and paper towel into dry waste containers.

E. What personal protection methods will be used to prevent external exposures.
Shielding __X__ (see below) Devices __X__ (see below)

Isotopes will be stored behind Lucite shields and all physical manipulations will be performed behind Lucite shielding. Gloves, lab coats and eye shields will be worn at all times. Pipetting devices and disposable tips will be used.

F. List the types of radioactive waste you will have and where you will store each form of waste until EH&S picks it up Estimate the volume per month: Short term experiments, once the experiments are completed we will have all waste and unused label removed by EH&S.

G. Protocols

VII. *In vivo* labeling of RNA and DNA by ^{3}H-Uridine and ^{3}H-Thymidine. This protocol is to be performed at the designated hot bench. All radioactive waste containers (liquid and dry), dedicated vacuum aspirator, microcentrifuge, water-bath and heating-block used exclusively for radioactive work are located at the hot bench. All equipment and radioactive waste containers are appropriately shielded and marked.

Radioactive ^{3}H-Thymidine or ^{3}H-Uridine label, (5μCi/ml) will be used to pulse 100 μl of exponentially growing *E. coli* cultures. Samples will be pulsed for 2 min. then chased with cold for 1 min. and then quenched with 5 mls of 5% Trichloroacetic acid (TCA). Labeling reactions are prepared a head of time by mixing label with 100μl of medium. Label is stored in the refrigerator until needed and is immediately returned to storage after the appropriate amount has been removed and all log sheets are completed. ^{3}H-Thymidine or ^{3}H-Uridine label (5 μCi/pulse) is added to 100 μl of sterile culture medium using a mechanical pipetting device and disposable tips (tips are disposed into dry waste). Enough labeling mix is made for each pulse in the experiment (total volume of labeling mix = number of pulses x 100 μl medium; total amount of label required = number of pulses x 25 μCi), 100 μl of the labeling mix is then transferred to reaction tubes (disposable tubes) and placed on ice until required. Any remaining labeling mix is removed and discarded into liquid radioactive waste (the pipette tip and labeling mix tube is discarded into dry waste). At the appropriate time interval 100 μl of the growing culture is added to the pulse tube, which has been placed in a water bath 2 minutes prior to use to warm the reaction mix to the appropriate temperature. The culture is allowed to incubate with the labeling mix for 2-5 minutes at the appropriate temperature (volume is 200 μl) at which point the reaction is chased with 2 μg/ml of cold Thymidine or Uridine for 2 minutes. The reaction is stopped by the addition of 1 ml of ice cold 5% TCA to precipitate the macromolecules and is then placed on ice until all samples have been taken. This is repeated for each time point. Once all time points have been taken, the quenched samples are then filtered using a multi channal vaccum chamber dedicated for this use. The filters are then washed 2 X with 2% TCA and finally with 1 ml of 90% ethanol. Filters are then air dryed and counted in the departmental scintillation counter.