{APPENDIX C}
PURPOSE AND PROCEDURES

Applicant Last Name: Schmoe, Ph.D. **Applicant First Name:** Joseph
Application Date: 11/18/2011

The following stipulations apply to all protocols (click here for an example of completed Appendix C):

Handling Procedures:

☒Yes ☐No My lab will use the personal protective equipment listed in Appendix A of this application when working with radioactive materials (at a minimum lab coat, gloves, eye protection).

☒Yes ☐No My lab will use shielding when appropriate.

- Plexiglas/Plastic for high/medium energy beta emitters (also useful for splash protection of lower energy beta emitters).
- Lead for Gamma or X-ray emitters.

Briefly describe procedures specific to the safe handling of radionuclides:
All procedures will be perfomed in the marked radioactive areas on absorbent pads behind Plexiglas shielding (for splash protection) using dedicated pipettes, pens, markers, etc.

Equipment:

☐Yes ☒No My lab will decontaminate the equipment listed in Appendix A of this application prior to non-radioactive use.
Briefly describe decontamination techniques in the procedures below.
The following equipment will be dedicated exclusively to radioactive materials use and labeled "radioactive": microcentrifuge, culture flasks, and pipettes. The following equipment will be decontaminated prior to non-radioactive use: stainless steel tongs, electrophoresis equipment, and water bath. Fumehoods, freezers and benchtops will be used for both radioactive and non-radioactive procedures and will have designated spaces for radioactivity. Surface decontamination will be done with Radiac Wash when possible, some equipment will be soaked in a solution of Radiac Wash if necessary. Following decontamination, confirmatory surveys will be performed on all equipment (using survey meter and wipe tests as appropriate).

Security/Food and Drink:

☒Yes ☐No My lab will ensure appropriate security for all radionuclides (including waste).
Briefly describe procedures specific to the security of radionuclides:
Stock vials will be stored in a padlocked refrigerator. Waste will be stored in a padlocked cabinet.

☒Yes ☐No My lab will identify areas outside of laboratory space for food and drink, application of cosmetics and contact lenses, etc.
State the location of the identified non-laboratory space:
Designated food and drink storage/consumption areas will be designated in the hallway outside the lab. Staff will be instructed to remove personal protective equipment and survey for personnel contamination prior to exiting the lab.

Radioactive Waste:

☐Yes ☒No My lab will segregate radioactive waste by radionuclide and physical form
If you checked no, explain:
Some experimental procedures require the use of both H-3 and C-14. We will segregate by physical form in these cases but the waste will contain both radionuclides.

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☒Yes ☐No My lab will transfer all radioactive waste to the Radiation Safety Office as specified in "Saint Louis University Waste Packaging Instructions for Laboratories" (available on the EHS website).

Surveys:

☒Yes ☐No My lab will conduct surveys according to the following schedule (please indicate weekly or monthly):
Surveys will include meter readings (unless only H-3 is being used) and wipe tests to be recorded in units of dpm/100cm^2.

☒Surveys will be performed during and after each use of radionuclides. These need not be documented.

☒For labs having > 100 microcuries of radioactivity in the aggregate in use or storage, documented weekly surveys will be performed.

☐For labs having < 100 microcuries of radioactivity in the aggregate in use or storage, documented monthly surveys will be performed.

For each radionuclide/radiochemical that you are applying, please provide the following information in the specified format (see sample protocol at the end of this application).

(A) Radionuclide
(B) Chemical Compound(s)
(C) Specify the purpose/type of experiment for which the requested radiochemical will be used.
(D) Provide a detailed by concise description, in narrative form, of procedures involving requested radiochemical.

C-14 Palmitate:

Purpose: Assay of palmitate oxidation

The procedure will be performed at room temperature under a fume hood. Palmitate oxidation will be assayed by trapping (in hyamine hydroxide) and scintillation counting of $^{14}CO_2$ produced by oxidation of [1- ^{14}C]palmitic acid. Cells will be incubated in culture flasks containing unlabeled palmitate (~500 µM) and 0.2 µCi/ml [1-^{14}C]palmitic acid. After addition of radiolabeled palmitate, flasks will be sealed with rubber serum tube caps with center well hanging buckets. Hyamine hydroxide (100 µL of 1 M solution in methanol) will be added to each hanging bucket using a syringe after inserting an 18-gauge needle through the rubber serum cap. After 1 h of incubation, the CO_2 production will be terminated by addition of 200 µL of 4 M H_2SO_4 to the incubation medium. The H_2SO_4 will be introduced with a small-gauge needle by poking through the rubber serum cap. Incubations will continue for another hour to allow trapping of CO_2 released from the medium. Hyamine hydroxide will be removed from the hanging buckets and placed in scintillation wells. Each hanging bucket will be washed with 100 µL of methanol, which will also be placed in a scintillation well. Media will be removed and stored in radioactive liquid waste, as will 0.9% saline used to wash the cells. Cells will be scraped in 0.3 M perchloric acid, and the perchloric acid homogenates will be assayed for protein content.

H-3: 2-deoxyglucose or 3-0-methylglucose

C-14: mannitol C-14: ascorbate:

Purpose: in vitro assay of glucose and dehydroascorbic acid transport rates in animal tissues (glucose and dehydroascorbic acid are transported by the same transporters)

i. Isolated animal tissues will be incubated for 10 or 20 min in 20 ml vials in shaking water baths in 2 ml of Krebs Heinseleit bicarbonate buffer containing 3 µCi/ml ^{3}H-labeled glucose analog and 0.3 µCi/ml ^{14}C mannitol. ^{14}C mannitol, that is not taken up by the tissues of interest, is used to measure extracellular space. Tissues will be removed, blotted, and clamp-frozen with tongs cooled in liquid nitrogen. Frozen tissues will be homogenized in 0.3 M perchloric acid (approximately 10 mg tissue/ml). Perchloric acid (PCA) extracts (100-200 µl) will be subjected to scintillation counting. Occasionally, PCA extracts will be assayed for glycogen or neutralized and then assayed for other metabolites (e.g. glucose 6-phosphate). Sometimes, tissue samples will be homogenized in a buffer containing protease and phosphatase inhibitors and subjected to western blotting.

ii. For some assays, ^{14}C ascorbate (l µCi/ml) will be used (in the presence of ascorbate oxidase to convert the ascorbate to dehydroascorbic acid) to assess dehydroascorbate transport.