GUIDELINES FOR USE OF SULFUR-35 AT MMRI

Projected In Vitro Uses, and ^{35}S Isotope conjugates.

Experiments will use either ^{35}S-conjugated nucleotide triphosphates, to label nucleic acids or examine nucleotide triphosphate mediated receptor/membrane binding studies, or ^{35}S–conjugated amino acids for protein labeling.

Nucleotide triphosphates.

Experiments involving ^{35}S–labeled nucleotide triphosphates may include cell-free, in vitro labeling of DNA or RNA. The most common experiments will include nucleic acid sequencing or in vitro transcription assays. Most experiments will use 50 uCi to 100 Ci per experiment. ^{35}S–labeled nucleotide triphosphates may also be used to examine the receptor-mediated/membrane association of nucleotide triphosphates.

^{35}S-conjugated nucelotide triphosphates are not volatile and experiments may be conducted on an unshielded bench.

Amino acids.

Experiments involving ^{35}S–labeled amino acids may include in vitro, cell-free protein labeling experiments, and cell culture experiments where protein labeling will occur following addition of labeled amino acids to live cell cultures.

As ^{35}S–labeled amino acids are examples of volatile forms of ^{35}S, all experiments involving ^{35}S–conjugated amino acids will include the use of free charcoal, or charcoal filters to absorb gaseous, volatile by-products, released from the opening of source vials (“source vials” may include the original shipping

container, or any vial/tube to which aliquots of radioisotope is distributed for prolonged storage.

Opening 35S Source Vials

1. Using a disposable lab coat, or disposable sleeves over a lab coat, ensure cuffs are covered by TWO pairs of gloves.
2. A source vial (whose lid contains a rubber septum) is transferred from the -20^{o}C freezer to a portable 23in x 17in x 14in enclosure, dedicated for opening ^{35}S vials (containing activated charcoal to ensure passive adsorption of volatile ^{35}S by-products if accidentally released from the vial; access to the enclosure is through two arm holes located on the front of the enclosure, the holes covered when not in use by a 1 mm sheet of transparent plastic).
3. While still frozen the septum is pierced with a cotton-plugged syringe needle or charcoal trap (Perkin-Elmer – catalog number NEX- 033T)(if cotton is used to plug a syringe needle, the cotton must first be combined with activated charcoal). **Care should be taken that the tip does NOT come in contact with the product.**
4. Quickly thaw at room temperature, or in a 37°C water bath. Any pressure developed will vent through the syringe needle.
5. Remove the needle and dispose of as contaminated equipment.
6. If the source vial is one obtained directly from the supplier, aliquots of ^{35}S-met or ^{35}S-cys will be transferred to polycarbonate or polystryene HPLC vials, which will be sealed with a rubber septum through which volatile by-products will be vented before opening the vial/tube, as outlined in steps 3-5 above (repeated freeze-thaws, and storage at temperatures below -20°C will result in rapid decomposition of product, e.g. the rate of decomposition is approximately 1% per week when stored at 4°C, with methionine being more susceptible than cysteine for conversion/decomposition.
7. Radioisotope may then be transferred to, and used on an open bench without shielding.

Cell-Free Translation/Protein Labeling Experiments

Cell free, in vitro translation experiments will generate ^{35}S-met or ^{35}S-cys (or ^{35}S-met **and** ^{35}S-cys) labeled proteins. Following appropriate opening of the source vial, ^{35}S-met or ^{35}S-cys may be used on an open bench with no shielding required.

Tissue Culture Translation/Protein Labeling Experiments

Aspects of cell-culture experiments requiring sterile handling of cell components will be conducted in a designated Biohazrd tissue culture hood, and will require the use of sterile activated charcoal-impregnated paper disks. Each disk fits a 100mm diameter tissue culture-grade Petri dish (VWR, catalog # *12574-070*). Free, unincorporated ^{35}S-met or ^{35}S-cys, given concentrations of amino acids in cell culture media, duration of protein labeling procedures, and elevated temperatures within an incubator, will produce ^{35}S-labeled volatile by-products at concentrations requiring removal to minimize the possibility of incubator contamination.

Following appropriate opening of the source vial, and addition of ^{35}S-labeled amino acid, a sterile disk is asceptically placed on the inner side of the Petri dish lid. As volatile by-products form from the free ^{35}-labeled amino acid, they will adsorb to the charcoal filter. Once the incubation/labeling period is complete, filters will be removed and stored in an ^{35}S-designated dry waste container to allow the isotope to decay.

For further information contact the MMRI Radiation Safety Officer or Alternate Radiation Safety Officer.