Standard Operating Procedure

Procedure	Uptake Assay using ^{3}H
Department	
Location	
SOP Prepared By:	

Section 1: Purpose

Uptake assays are a tool to measure the incorporation of a specific molecule. In principle, a radioactively tagged molecule is fed to a culture of growing cells or organisms and allowed to incubate, allowing the cells to take up the molecule. After a period of time, the cells are lysed and purified so the amount of radioactivity can be measured. This can provide useful information into the processing mechanisms of the particular cell or organism in question.

This assay has numerous uses for studying different molecules that are tagged with different isotopes. ^{3}H can be used to study the uptake of key molecules in cells or microorganisms. At the University, labs are using a radioactive hypoxanthine, a precursor for DNA synthesis, to see if and how certain drugs interfere with the synthesis of DNA[1,2]. ^{3}H can also be a label for a form of glucose to determine the effect of certain molecules, like insulin, on glucose uptake[3,4].

Section 2: Personal Protective Equipment and Survey Equipment

PPE:

- Lab coat
- Nitrile Gloves
- Heat resistant gloves
- Safety Glasses
- Closed-toe shoes

Survey Equipment:

- Scintillation counter for swipe tests

Section 3: Radioactive Material

^{3}H
Common forms used in the labs: ^{3}H-hypoxanthine, ^{3}H-2 deoxy-D-glucose (Note: they are used separately for different research)
Supplier: PerkinElmer
Starting Activity: 50 uCi/mL (hypoxanthine),1 mCi/mL (glucose)
Typical use quantities:1.5 mL for 96-well plate (hypoxanthine), 50 uL per well (glucose)

Activity used per experiment: _____
RAM handling time: _____
Frequency of experiment: _____

Section 4: Potential Hazards

- ^{3}H is a low-energy beta emitter and has a half-life of 12.4 years. ^{3}H is also harder to detect due to its low energy.
- Cell cultures/microorganisms may present a risk to human health and the environment.
- Phosphate-buffered saline solution (PBS) is used at low concentrations for some of the washes. There are several hazards associated with PBS.

Any other hazards (potential for airborne release of radioactive material, chemical hazards, etc.)?

Section 5: Safety Precautions

The following precautions should be taken while handling ^{3}H:

- Designate area for handling ^{3}H (and RAM) and clearly label all containers and equipment.

Equipment used with RAM used in this procedure:

  - Pipettes
  - Aspirator
  - Centrifuge/microcentrifuge
  - Incubator
  - Freezer
  - Scintillation Counter
  - Plate reader

- Use filtering pipette tips to prevent contamination of pipette.
- Line all RAM countertops with absorbent sheets.
- Pulse centrifuge the tubes to contain the liquid mix at the bottom of the tube.
- Survey all areas and equipment where RAM is used with a swipe test before and after the procedure.
- Minimize exposure by keeping a hot hand (holding tubes with radioactive material) and cold hand (pipetting, etc.) as much as possible.
- Comply with all biosafety rules and regulations on the proper handling of cell cultures/microorganisms. Research may require certification as an upper level biosafety lab.

Consult the MSDS for PBS and follow the proper handling and procedure in case of an accident[3].

Section 6: Procedure

+indicates step should be conducted in a designated biohazard fume hood if necessary. Regulations may vary depending on the cell culture or microorganism used.
**indicates step should be conducted in a designated RAM area*
The following procedure has been generalized to include different types of uptake assays.

A. Preparation of Cell Culture/Microorganism

1) +Prepare a cell culture or plate with microorganisms in a vessel[2] in a designated cell culture hood using the lab's specific procedures.
    a) Cell culture vessels include petri dishes, flasks, roller bottles, etc.
2) *+If necessary, allow cells/microorganism to starve for a variable amount of time, ranging from overnight - 48 hrs.*
    a) *The starvation step is necessary for applications that study the importance of a certain nutrient in the system.*
3) *+If necessary, additional steps to wash or treat cells with a substance might be performed after starvation. Plates are incubated for a number of hours in incubator.*
4) +Distribute cells onto a multi-well plate or in Eppendorf tubes with a pipette or multichannel pipette.
    a) Multi-well plates are used for many replicates, whereas Eppendorf tubes are good for a larger number of cells.

B. Addition of Radioactively labeled molecule

5) +*Move to a designated RAM area (if not already under a fume hood). Add an appropriate volume and concentration of radioactively labeled molecule into the wells/tubes.
6) +*Incubate cells to allow them to take up the radioactive molecule for a certain amount of time and temperature.
    a) The time ranges from several minutes - one day, and is quite variable depending on the test.
    b) The temperature ranges from room temperature to 37 C. Keep samples in a closed cabinet or incubator.

There are two main methods of processing the cells and detecting counts. The most common technique follows steps C-D. Steps E-F describe a more outdated method for processing and detecting radioactivity on multi-well plates.

C. Processing of Cells

7) *+*If necessary, spin down cells in a RAM designated centrifuge for a few minutes, and aspirate off the medium from the tubes/wells.*
8) *+*If necessary, add ~100 uL of low concentration of Trypsin using a RAM designated pipette, which breaks down proteins that help the cell adhere to the surface of the tubes. Let tubes sit for several minutes at room temperature. Quench trypsin reaction using a special medium.*
9) +*Wash cells on ice using ice cold phosphate-buffered saline (PBS, concentration varies). Repeat 2-3 times.
10) Centrifuge for 2 minutes, then aspirate off supernatant. Repeat three times.
11) +*Lyse cells by adding a lysis buffer with a pipette. Some common buffers include low concentrations of SDS and Triton X-100.
12) +*Mix and incubate if needed. Use a microcentrifuge to pull the suspension down to prevent contamination.
13) For ^{14}C studies, the addition of chloroform may required. See lab protocols for further details[1,2].

D. Activity Counting Using Scintillation Counter

14) Prepare scintillation vials with an appropriate volume (usually in mL) of scintillation fluid.
15) *Transfer an appropriate volume (around 100 uL - 250 uL) of the suspensions from each tube to a scintillation vial and swirl until clear.

16) *Place the vials in the scintillation counter and analyze counts using computer software.

E. Processing of Cells and Transfer Using a Cell Harvester

17) *After incubation, wrap multi-well plate in aluminum foil and freeze in a -80 degree freezer to lyse cells.
18) *Thaw cells at room temperature after freezing unwrapped.
    a) The freeze-thaw process kills the microorganism, so a fume hood is no longer necessary.
19) * Insert the multi-well plate into a cell harvester to transfer the cell components onto a disposable filter plate. Allow the filter plate to dry overnight or in a designated oven.

F. Activity Counting Using Plate Reader

20) Add an adhesive backing to the filter plate.
21) Stick the filter plate onto a plate reader, and close the lid.
22) Analyze the counts by running the plate reader software.

Section 7: Spills/Incidents/Clean Ups

- For spills onto lined countertops, carefully discard of the absorbent lining into the solid RAM waste box. Check the countertop with a swipe test if needed.
- Check the centrifuge and scintillation counter for possible leakage. If contaminated, clean the rotor of the centrifuge - use an effective cleaner for radioactive material. Check using swipe test.
- Conduct a swipe test on the cell harvester and plate reader (if applicable). Clean equipment with an effective cleaner.
- For major spills or any personal contamination, contact Radiation Safety Services for proper instructions and guidance. Try and contain the spill and check yourself and the area for radioactivity.
- For small spills onto lined countertops, carefully discard of the absorbent lining into the solid RAM waste box. Check the countertop with Geiger counter afterwards. Document the spill and cleanup procedure (https://www.ehs.harvard.edu/node/7589) used with other radiation records and notify radiation_protection@harvard.edu.
- At any point you may call Radiation Safety Services for assistance.

Section 8: Transportation, Storage, and Disposal

- Store ^{3}H stock in a locked -20^{o} Celsius RAM-designated freezer in a locked box. Keep key/passcode in a safe place.
- If transportation of samples containing ^{3}H is necessary (for example, from the fume hood to scintillation counter), place samples in a secondary container to prevent spills onto the floor.
- When using a pipette that involves possible contamination of RAM, keep the lid of the solid RAM waste container slightly open to quickly discard of pipette tips.
- Small volumes of possibly radioactive liquid, may be poured onto paper towels inside the solid RAM waste container.
- Liquid from aspiration is collected in the flask (trap). Dispose of liquids down the sink, and check to make sure the amount disposed is under the disposal limit for ^{3}H.
- Radioactive waste should be tagged and separated by isotope.

Section 9: References
● Harvard EHS website: https://www.ehs.harvard.edu/services/radiation-protection ● MSDS PBS: http://www.uccaribe.edu/research/wp-content/uploads/2014/03/Phosphate-buffered-saline.pdf ● Cocktails for Liquid Scintillation Counting: https://www.perkinelmer.com/lab-solutions/resources/docs/APP_Cocktails-for-Liquid-Scintillation-Counting-011940_01.pdf