Standard Operating Procedure

Procedure	Radioimmunoassay with ^{125}I
Department	
Location	
SOP Prepared By:	

Section 1: Purpose

Radioimmunoassays are used for detecting the concentration of a specific antigen or substrate in samples using antibodies. Samples may be obtained from outside or ordered from a company. The substrate in the sample and a radioactively labeled version of the substrate are placed in the same tube. When the antibody is added, the two forms of substrate compete to bind to the antibodies. The unbound substrates are washed, and the amount of radiation is detected in a gamma counter. Higher counts indicate less antigen in the serum.

This technique is sensitive and uses a very small amount of ^{125}I, which is supplied in commercial RIA kits. Standards of different concentrations of pure, unlabeled substrate are prepared so the results can be compared against a standard curve. An application of this procedure includes studying levels of Human C-peptide in different populations from urine samples[1]. The ^{125}I labeled Human C-peptide as well as the antibody is supplied in a kit[2].

Section 2: Personal Protective Equipment and Survey Equipment

PPE:

- Lab coat
- Nitrile Gloves
- Safety Glasses
- Closed-toe shoes

Other Equipment:

- Geiger counter with pancake probe
- Personal chest dosimeter
- (Note: a ring dosimeter should be worn when working with amounts greater than 1mCi of radioactive material)

Section 3: Radioactive Material

Note: The labeled substrate depends on what kit is being used and what research is being conducted.

^{125}I labeled peptide of interest
Supplier: RIA Kit, EMD Millipore

Activity: 100-700 uCi/ug (varies by kit)
Typical use quantities: no more than 3-5 uCi (varies by kit)

- Radioactive material (RAM) benchtop exposure time: 5-10 minutes

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

Section 4: Potential Hazards

- ^{125}I is radioactive and a gamma ray emitter.
- Reagents supplied in the kit may be hazardous. These reagents include:
  - 0.08% sodium azide in assay buffer and precipitating reagent
  - Standard substrate may be hazardous (depends on the substrate).
  - ^{125}I labeled substrate is harmful to the environment, dangerous if swallowed and may cause serious eye damage.
  - Antibody specific to tracer may cause serious eye irritation.
  - Precipitating reagent may cause serious eye irritation.
- Serum/sample containing the substrate of interest may come from human samples.

Section 5: Safety Precautions

The following precautions should be taken while handling radioactive materials (including ^{125}I):

- Designate area for handling radioactive materials and clearly label all containers and equipment.
- Line all RAM countertops with absorbent sheets.
- Survey all areas and equipment where RAM is used with a Geiger counter before and after the procedure.
- Survey hands, body, and face with Geiger counter after conducting the procedure.
- Minimize exposure by keeping a hot hand (holding tubes with radioactive material) and cold hand (pipetting, etc.) as much as possible.
- Perform all non-radioactive steps beforehand.
- Consult the MSDS for specific reagents used in the assay (can be found in on supplier's website). General precautions are listed below:
- Comply with regulations in place on how to handle and dispose of sodium azide.
- Consult the RIA kit's manual to check if the substrate standard is hazardous and note precautions.
- Wear eye protection and do not release ^{125}I labeled substrate into the environment.
- If the tracer, antibody or precipitating reagent gets into eyes, rinse eye carefully with water for several minutes, and remove contact lenses if present and are able to do so.
- Be aware and informed of any hazards regarding the samples containing the molecule of interest.

Consult the MSDS for reagents used in the procedure and follow safety instructions accordingly.

Section 6: Procedure

**Indicates step should be performed in a designated RAM area*

The following procedure follows the procedure given in an RIA kit from EMD Millipore. The assay is performed in duplicates of each sample which ensures reliability and consistency. Labs may choose to follow another protocol; however the steps should be the same in principle.

A. Preparation of Standard Samples and Controls (to compare against substrate levels from the samples). All materials provided in kit.

Standard Curve is as follows: Total Count (TC),Non-specific binding, Reference, Standards (0.156 - 2.5 ng/mL)

1) Label two 12x75 borosilicate glass tubes for each control below and add the given volume of buffer/reagent in duplicate using a pipette:
    a) TC tubes: no volume added, blank tubes
    b) Non-specific binding tubes: add 200 uL of Assay Buffer only
    c) Reference tubes: add 100 uL of Assay Buffer only
    d) Quality Control 1 tubes: add 100 uL of quality controls 1
    e) Quality Control 2 tubes: add 100 uL of the quality control 2
2) Label five 12x75 borosilicate glass tubes 1-5 as the standard tubes.
3) Add a given volume of Assay Buffer to each tube using a pipette.
4) Perform a 1:2 serial dilution with the standard substrate 5 times.
    a) For example, if 1 mL of Assay Buffer was added to each tube, then 1mL of the standard substrate from the kit should be added to Tube 1 and vortexed, 1 mL from Tube 1 should be transferred to Tube 2 and vortexed, 1 mL from Tube 2 should be transferred to Tube 3, etc.
    b) Vortex in between transfers, and use a new pipette tip for each transfer.
    c) Starting concentration of standard substrate: 5 ng/mL
5) Label 12 more 12x75 tubes in duplicate so that each standard has two tubes.
6) Aliquot 100 uL of each standard to its set of tubes.

Tube	Concentration of Standard Substrate
Tube 1	2.5 ng/mL
Tube 2	1.25 ng/mL
Tube 3	0.675 ng/mL
Tube 4	0.313 ng/mL
Tube 5	0.156 ng/mL

B. Preparation of assay with substrate from samples

7) Label two empty 12x75 tubes for each sample.
    a) The number of samples will vary depending on how many samples were previously collected from the study.
8) Dilute each sample with assay buffer in another 12x75 tube (exact measurements vary).
9) Transfer 100uL of the diluted sample into the two tubes labeled for that particular sample. Repeat for the rest of the samples.

(Note: The volume in the control, sample, and standard tubes does not necessarily have to be 100 uL but should be consistent with the volume of tracer added in the next step.)

C. Addition of Radioactively Labeled Substrate (tracer)

10) *Reconstitute tracer with Label Hydrating Buffer provided in the kit 30 minutes before using.
11) *Add 100 uL of tracer from the kit to all control, sample, and standard tubes using a designated RAM pipette.
12) *Add 100 uL of antibody from the kit to all tubes except for the Total Counts and non-specific binding tubes.
13) *Close each tube with a cap? and vortex each tube briefly.
14) *Incubate the tubes overnight at 4 degrees C in a refrigerator on a tube rack. Cover the tubes with parafilm and aluminum foil.

D. Precipitation and Detection (Day 2)

15) *Take the samples out of the refrigerator, and add 1 mL of cold (4 degree C) precipitating reagent (secondary antibody) from the kit.
16) *Vortex tubes and incubate for another 20 minutes in 4 C.
17) *Centrifuge tubes (except for total counts tubes) at 4 C for a period of time until a firm pellet is formed at the bottom of the tubes.
18) *Pour off supernatant down the sink, then inverting onto a stack of paper towels.
    a) If there is still liquid, use an aspirator to vacuum the remaining liquid.
19) *Make sure tubes are arranged in an order that is easy to analyze
20) *Place tubes in a gamma counter and run the software to count the amount of radioactivity in each sample.

Section 7: Spills/Incidents/Clean Ups

- 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. Check the centrifuge for possible leakage of radioactive material from tubes. If contaminated, clean the rotor of the centrifuge with an effective cleaner for radioactive material. Check again with Geiger counter, and keep cleaning until counts are at background level.

- For possible leakage of radioactivity in the gamma counter or on the vortex, clean with an effective cleaner and check with Geiger counter.
- At any point you may call Radiation Safety Services for assistance.

Section 8: Transportation, Storage, and Disposal

- Store ^{125}I stock in a locked box in a locked fridge or freezer.
- For short term storage of the reagents from the kit, store in a 4 C fridge. For long term storage of the reagents, store in a -20 C freezer.
- Transport samples containing ^{125}I in secondary containment to prevent spills directly onto the floor.
- Keep the lid of the solid RAM waste container slightly open to quickly discard of pipette tips.
- The main disposal method for the RIA is sink disposal. Log the amount of ^{125}I that is disposed in the sink each time the assay is performed.
- Small volumes of possibly radioactive liquid may be poured onto paper towels inside the solid RAM waste container.
- Wash glass tubes and pipette for the tracer with ethanol, and check with a Geiger counter.
- Store reusable glass tubes in a bag in a labeled cabinet to allow the isotope to decay.
- Radioactive waste should be tagged and separated by isotope.

Section 9: References

- Harvard EHS website: https://www.ehs.harvard.edu/services/radiation-protection
- RIA Human C-peptide Kit and protocol (and other RIA kits): https://www.emdmillipore.com/US/en/product/Human-C-Peptide-RIA,MM_NF-HCP-20K