Standard Operating Procedure

Procedure	In vitro translation with ^{35}S-methionine
Department	
Location	
SOP Prepared By:	

Section 1: Purpose
In vitro translation is a useful tool for studying protein interactions and may be coupled with other procedures. A DNA or RNA template is mixed with a cell-free translation system and radiolabeled ^{35}S methionine, resulting in the synthesis of radiolabeled protein. This protein may then be used in other assays to detect protein transport[1] or protein-protein interactions[2], which are analyzed by common imaging techniques such as phosphorimaging.
Section 2: Personal Protective Equipment and Survey Equipment
PPE: • Nitrile Gloves • Lab coat or lab gown • Proper enclosed shoes • Safety glasses Other Equipment: • Geiger counter • Personal chest dosimeter
Section 3: Radioactive Material
^{35}S methionine Supplier: Perkin Elmer Starting Activity: 10 uCi/uL Typical use quantities: ~2 uL per experiment **Activity used per experiment:** _____ **RAM handling time:** _____ **Frequency of experiment:** _____
Section 4: Potential Hazards
• ^{35}S is a radioactive beta emitter. • ^{35}S labeled amino acids seem to have a volatile component, which may be water soluble.

- A few risks are associated with Rabbit Reticulocyte Lysate, a common cell-free translation system.

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

Section 5: Safety Precautions

The following general precautions should be taken while handling ^{35}S:

- Designate area for handling ^{35}S and clearly label all containers and equipment. Equipment used in this procedure:
  - Pipette
  - Ice bucket
  - Fume hood
  - Centrifuge
  - Heat block
- Prepare the workspace before conducting the actual procedure to maximize efficiency and keep radioactive exposure to a minimum.
- Use filtering pipette tips to prevent contamination of pipette.
- 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.
- ^{35}S methionine delivery vials and thawed materials should be opened in a fume hood. Vials of ^{35}S methionine should be opened and used in ventilated enclosures.
- To prevent water contamination of the volatile component of ^{35}S methionine, use a heat block for incubating samples instead of a water bath. The incubation temperature is not hot enough to produce potential steam or water vapor from the samples.
- Conduct all non-radioactive steps of the procedure before moving to the RAM corner.
- Minimize the time of contact with ^{35}S methionine by setting up bench space before starting procedure.

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

Section 6: Procedure

**indicates step must be performed in a designated RAM area*
 # indicates step must be performed under a ventilated hood

There are in vitro translation kits that come with lysate, buffer, non-radioactive amino acid mix minus methionine, and nuclease-free water. If starting with RNA, Ambion's Retic Lysate IVT Kit (standard is sufficient to perform translation. If starting with DNA, Promega's TNT® Coupled Reticulocyte Lysate System has the necessary ingredients to perform transcription and translation in the same tube[5].

In Vitro Translation Reaction (ingredients needed in uL amounts, total volume ~50 uL)

1) Thaw components from kit and other materials slowly on ice.
2) Add together components from kit in an Eppendorf tube. (May be helpful to include a control reaction in another Eppendorf tube containing none of the DNA or RNA to measure background incorporation of amino acids)
    a) Lysate (~35uL if using standard kit, less if using coupled translation kit)
    b) Buffers
    c) RNap
    d) Amino acids minus methionine

(actual volumes will vary according to kit, but usually not more than 2uL of ^{35}S methionine is used)

3) Add the appropriate volume (in uL) of the purified RNA or DNA sample.
    a) There are several kits available for DNA or RNA isolation. When isolating RNA, it is essential that RNase free tubes and water are used to prevent RNA degradation.
4) Optional: Add approximately 1uL of RNasin Ribonuclease Inhibitor (not supplied in kit, ordered separately) to the mix to prevent RNA degradation. Mix well with pipette tip and centrifugate as needed.
5) *# In the RAM area, take out the ^{35}S labeled methionine stock from the locked fridge. Add the appropriate volume of ^{35}S methionine to tube. Mix well with pipette tip and centrifugate as needed.
6) *Add dH_2O up to 50 uL to sample tubes. Mix well with pipette tip and pulse centrifugate as needed.
7) *Immediately incubate samples at 30^o Celsius for 45-90 minutes on a heat block.

Steps 4-6: *When adding components into tubes, pulse centrifugation helps pull down the liquid to the bottom of the tube. This is especially helpful when the radioactive isotope is added so exposure is minimized.*

Analysis of Results:

Simple analysis techniques to detect the radiolabeled protein are described in the Technical Manual from Promega[4] (SDS PAGE, incorporation assay, etc). The results of in vitro translation may also be used in further research that requires more in-depth procedures that are unique to the lab. Regardless, all procedures after the in vitro translation step must be performed in a RAM area.

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. If contaminated, clean the rotor of the centrifuge - use an effective cleaner for radioactive material. Check again with Geiger counter, and keep cleaning until counts are at background level. Document the spill and cleanup procedure 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 ^{35}S methionine stock in a locked 4 degrees Celsius fridge preferably in a locked acrylic box. Keep key/passcode in a safe place.
- If transportation of samples containing ^{35}S methionine is necessary, for example, from the hood to the bench space, place samples in a secondary containment to avoid direct spills onto the floor.
- Small volumes of possibly radioactive liquid may be poured onto paper towels inside the solid RAM waste container.
- Wash buffers in the hybridization step can be discarded in the sink as long as the amount of ^{35}S is below the daily sink disposal limit of 100 uCi.
- Check ice from the ice bucket for any radioactive contamination with a Geiger counter. If there is no contamination, ice may be disposed in a regular lab sink.
- Radioactive waste should be tagged and separated by isotope.

Section 9: References

- Harvard EHS website: https://www.ehs.harvard.edu/services/radiation-protection
- Cell-free systems for in vitro translation: https://www.thermofisher.com/us/en/home/references/ambion-tech-support/large-scale-transcription/general-articles/the-basics-in-vitro-translation.html
- Rabbit Reticulocyte Lysate System Technical Manual: https://www.promega.com/-/media/files/resources/protocols/technical-manuals/0/rabbit-reticulocyte-lysate-system-protocol.pdf
- TNT® Coupled Reticulocyte Lysate System: https://www.promega.com/products/protein-expression/eukaryotic-cell-free-protein-expression/tnt-coupled-reticulocyte-lysate-systems/
- TNT® Coupled Reticulocyte Lysate System manual: https://www.promega.com/-/media/files/resources/protocols/technical-bulletins/0/tnt-coupled-reticulocyte-lysate-systems-protocol.pdf
- MSDS Rabbit Reticulocyte Lysate: https://tools.thermofisher.com/content/sfs/msds/2011/F1200G_MTR-NALT_EN.pdf
- RNasin Ribonuclease Inhibitor: https://www.promega.com/resources/protocols/product-information-sheets/n/rnasin-ribonuclease-inhibitor-protocol/