Standard Operating Procedure

Procedure	Nucleic acid end labeling using ^{32}P
Department	
Location	
SOP Prepared By:	

Section 1: Purpose

Nucleic acid end labeling involves the removal of the end phosphate groups of a nucleic acid sequence and the substitution of ^{32}P-labeled phosphate groups. The result of end labeling (as well as *in vitro* transcription) is a radioactively labeled DNA or RNA fragment that can be used as a tool in further research (ex. Uptake assays, Northern Blotting). Commercial kits, such as Ambion's KinaseMax kit, may be used to perform this procedure, which has the enzymes and buffers (excluding the radiolabeled ATP) necessary for the end-labeling reaction.

Section 2: Personal Protective Equipment and Survey Equipment

PPE:

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

Survey Equipment:

- Geiger counter with pancake probe
- Personal chest dosimeter
- Personal finger dosimeter

Section 3: Radioactive Material

ATP, [γ -^{32}P]
Supplier: Perkin Elmer
Starting activity: 10 uCi/uL
Typical use quantities: for commercial kit about 50 uCi per reaction (20 uL). An equation to determine the amount needed for a reaction is provided in the kit manual.

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

Section 4: Potential Hazards

- ^{32}P is a high-energy beta emitter and has a half-life of 14.29 days. ^{32}P can present a substantial skin and eye dose hazard.
- Reagents or buffers used in the procedure may present hazards.

Section 5: Safety Precautions

The following precautions should be taken while handling ^{32}P:

- Designate area for handling ^{32}P and clearly label all containers and equipment. Equipment used in procedure:
  - Pipettes
  - Heat block or thermocycler
- Prepare the workspace before conducting the actual procedure to maximize efficiency and keep radioactive exposure to a minimum.
- Any samples, stock, or equipment containing ^{32}P should be used behind Plexiglass shielding.
- 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 Geiger counter before and after the procedure.
- Keep Geiger counter on when working with ^{32}P directly.
- 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.

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

Section 6: Procedure

**indicates step must be performed in a radioactive area and behind Plexiglass shielding.*

The following procedure closely follows the procedure from Ambion's KinaseMax kit. However, any other developed procedures should keep exposure to radioactivity at a minimum. The general equipment and reagents used should be similar if using another protocol.
Volumes in parentheses are for a 20 uL reaction.
If working with RNA, use RNase-free Eppendorf tubes to prevent RNA degradation.

A. Removal of phosphate groups

1) Obtain DNA or RNA template of interest using isolation and purification techniques.
2) Pipette non-radioactive components - DNA/RNA substrate, 10X dephosphorylation buffer, and calf intestine alkaline phosphatase (1 uL of each, 0.1-25 pmol of DNA/RNA)- in appropriate volumes on a separate bench in an eppendorf tube. Add nuclease-free water up to the appropriate volume (10 uL) with a pipette.

a) The calf intestine alkaline phosphatase should be used only if working with DNA or RNA (not oligonucleotides)

3) Incubate the reaction at 37° C on a heat block or thermocycler for about 1 hour.
4) Transfer an appropriate volume (10 uL) of well-mixed phosphatase removal reagent (PRR). Allow the tube to incubate at room temperature for a few minutes, flicking the tube occasionally.
5) Remove the phosphate removal reagent by centrifuging for a few seconds until the PRR becomes a pellet. This is done so the PRR does not inhibit the kinase reaction in the next steps.
6) Transfer the supernatant into a new tube.

B. Kinase Reaction

7) *Move to a RAM designated area.
8) *Take out the [γ -32P]-ATP and add the appropriate volume (~5 uL) to the mix with a pipette. Mix well.
9) *Add the other reagents - unlabeled ATP, 10X kinase buffer, and T4 Polynucleotide Kinase (1-2uL) - to the mix with a pipette. Mix by pipetting up and down.
10) *Incubate the reaction mix on a heat block or thermocycler at 37 C for about 1 hours.
11) *Stop the reaction by adding EDTA (for RNA) and heating to 95° C for 2 minutes on a heat block or in a thermocycler.

C. Further Applications

12) *Depending on research's application, the resulting DNA or RNA transcript can be purified and/or quantified using various detection methods (scintillation counter, gel electrophoresis and phosphorimaging, etc.)

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 kit reagents in a -20 degree C freezer. Nuclease-free water can be stored in at room temperature, a fridge, or freezer.
- Store [α-^{32}P]-UTP stock in a locked 4 degrees Celsius fridge in a locked acrylic box. Keep key/passcode in a safe place.
- If transportation of samples containing ^{32}P is necessary, place samples in an acrylic container as a secondary containment.
- When using a pipette, keep the lid of the solid RAM waste container slightly open to quickly discard

pipette tips.

- Small volumes of possibly radioactive liquid may be poured onto paper towels inside the solid RAM waste container.
- Dispose of gels and membranes (if applicable) in the solid RAM waste container. If the gel still has some liquid, discard in a RAM waste container with sawdust.
- Radioactive waste should be tagged and separated by isotope.

Section 9: References

- Harvard EHS website: https://www.ehs.harvard.edu/services/radiation-protection
- Ambion KinaseMax 5' End-labeling kit (protocol, manual, and MSDS available) : https://www.thermofisher.com/order/catalog/product/AM1520