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THE UNIVERSITY OF ARIZONA
RESEARCH, INNOVATION & IMPACT
Core Facilities

Imaging Cores – Electron

General SOP Guidelines for Samples Submission

1. Primary Fixation of Samples

Glutaraldehyde 2.5 %, or a solution of Paraformaldehyde 2 % and Glutaraldehyde 2.5 % in 0.1 M PIPES or PBS pH 7.4 is recommended as a primary fixative, and depend on the biological material.

1.1. Cells Culture Samples

- Cell culture media is removed from culture plate and rinses 3 times with 0.1M PIPES or PBS buffer pH 7.4.
- Fixation of the culture plate with glutaraldehyde 2.5 % in 0.1M PIPES or PBS buffer pH 7.4 for 30min to 1hr.
- Rinse 3 times with the buffer.
- Scrap off the cells from the plate in buffer, spin down the cells and pellet at 1000g for 5 minutes in 1.7ml Eppendorf tube.
- Please make sure to submit to the EM facility at least 1 million cells or a visible pellet.
- Submit to the Core facility in 0.1 M PBS pH 7.4 buffer.

1.2. Tissue Samples

- Tissues should be removed from the animal as quickly as possible postmortem and immersed in the primary fixative during dissection into EM-sized pieces.
- The tissue samples for EM should be ~ 1 – 2 mm^3.
- The fixative fluid should be approximately 5 times the volume of the sample.
- Samples should not be taken from large pieces of previously fixed tissues further than 1 mm from the surface.
- Sample surfaces should never be allowed to dry.

2. Procedure

- **Necropsy:** During necropsy, quickly cut off a small piece of fresh tissue several mm in thickness and place it into a large droplet of fixative in a Petri dish. Slice the tissue sample with a razor blade into pieces 1- 2 mm^3. Determine if a long dimension is needed for sample orientation. Prepare 3 fragments per tissue sample and handle them by touching with an applicator stick and place them into a 2 - 4 mL vials containing 1–2 ml of fixative.

- **Skeletal Muscle.** Cut long, thin superficial slices from muscles and hold them in clamps or carefully pin them in a cork or sylgard dish prior to primary fixation. Failure on doing this will lead to contraction during fixation. Each sample should be flat embedded, with 2 blocks oriented for longitudinal sections and 2 blocks oriented for transverse sections. If there is limited sample quantity, make the longitudinal blocks first, because they are generally the only ones examined.

- **Heart Muscle.** The wall of a heart can be oriented as described for skeletal muscle. Attaching the sample to pins prior to primary fixation will prevent contraction. Orientation should be performed to obtain longitudinal and transverse sections.

- **Brain, kidney, and liver.** Perform fixation in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M dimethyl arsenic acid sodium buffer (cacodylate buffer), 0.1 M PIPES or PBS pH 7.4, overnight, or alternatively 24 h with one exchange of the fixative solution. For Peripheral Nerves, orient long sections

of tissue for transverse sections and for Central Nervous System, random orientation is usually adequate.

- **Eyes.** For best fixation of eye tissue, the eye is incised on 1 side, top to bottom, midway between the cornea and optic nerve. The entire eye should then be quickly placed into a container with 10X more fixative than the eye volume. Specific areas of interest should be dissected out after primary fixation, but before further processing since tissue blackening after osmication makes identification of specific areas in the eye difficult.

3. Suspension Samples for TEM

If the sample consists of suspended biological material (e.g. virus, bacteria, yeasts, protozoans, etc.) agarose-embed them after washing out the primary fixative with buffer.

4. Suspension Samples for Negative Staining

Biological material as before, including exosomes, liposomes, or proteins, need to be prepared at a concentration suitable for negative staining. Nanoparticles and nanocellulose can also be prepared unstained for direct observation.

5. Powder Samples

If the sample is in the form of a powder, this will require to be solubilized in molecular grade water or buffer to obtain a suspension suitable for unstained or negative staining grids preparation.

6. Sample Storage

If you need to store the samples after the primary fixation and before processing, store them in the buffer overnight at 4 °C.

7. Buffers

For most samples, 0.1 M PIPES or PBS pH 7.2 - 7.4 for the tissue/cells is recommended.

8. Samples Labeling

All samples need to be identified and labeled, accordingly.