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🌐 Sample fixation of biopsy tissue for Electron Microscopy (EM)

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Protocol status: Working

We use this protocol in our group and it is working for traditional electron microscopy. This protocol may need modification for specific electron microscopy techniques, such as correlative light and electron microscopy (CLEM) or when perfusion of animals is possible.

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Keywords: electron microscopy, sample fixation, sample fixation of biopsy tissue, entire electron microscopy workflow, crucial step in the entire electron microscopy workflow, biopsy tissue, optimal ultrastructure preservation, fixation time, tissue, cellular ultrastructure, precious human tissue, clinical setting, preservation

Abstract

The most crucial step in the entire electron microscopy workflow is sample fixation. Tissue needs to be preserved in strong fixative as soon as possible to maintain cellular ultrastructure. In a clinical setting, fixation time is critical in order to capture precious human tissue adequately. Of course, priority is given to patient care, and therefore, the tissue sometimes cannot be handled as quickly as needed for optimal ultrastructure preservation. However, we recommend 2 minutes as a “best practice” time to start of preservation.

Citation

Karnovsky, Morris J.. A Formaldehyde-Glutaraldehyde Fixative of High Osmolality for Use in Electron Microscopy. Journal of Cell Biology.

Troubleshooting

Safety warnings

! Researchers are advised to wear safety glasses, lab coats, and gloves when handling fixative.



- 1 In order to facilitate quick fixation, the team of clinical coordinators should be provided with Eppendorf tubes containing 1.5 mL of fixative solution to have on-hand in the operating room during biopsies using 18-gauge core needles where 3-4mm of core is preserved. Larger volumes may be required for resections.
- 2 Karnovsky's fixative (2.5% paraformaldehyde, 2.5% glutaraldehyde in 0.1M Na Cacodylate buffer (pH 7.4)) is the solution of choice (Karnovsky, 1965).
- 3 Tissue should be placed gently into the Eppendorf tube, ensuring that the tissue is completely submerged in fixative solution to prevent drying out. Use of forceps should be avoided whenever possible to minimize mechanical damage. An orange stick or scalpel blade can be used to "scoop" the tissue into the tube.
- 4 Eppendorf tubes with and without tissue are stored at 🌡️ 4 °C.

2m

Citations

Karnovsky, Morris J.. A Formaldehyde-Glutaraldehyde Fixative of High Osmolality for Use in Electron Microscopy