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Sample fixation of biopsy tissue for Electron Microscopy (EM)

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1 *Works for me* dx.doi.org/10.17504/protocols.io.4bigske

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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.

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

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[2m] 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** .

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