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Tissue Fixation Preparation

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes fixation of tissue in preparation for pathology review to assess tissue quality. Prior to performing molecular analyses, it is critical to have experienced pathologist confirm the tissue of interest is captured, the region of interest is represented and there are no abnormalities. Fixed tissue is preferred for pathologist review as it prevents autolysis and degradation of the tissue, cells and cellular components so they can be observed both anatomically and microscopically in their most natural state following sectioning. Various reagents can be used for fixation. Formaldehyde is a water-based solution and is the most commonly used chemical fixative. Paraformaldehyde has been found to be a preferred fixative for the ovary as it maintains oocyte and follicular morphology which is often disrupted with other fixatives.

Materials

- Freshly dissected tissue block
- Ice
- 70% EtOH (made fresh; kept at 4°C)
- Ice bucket
-  1.5 mL LoBind tubes **Eppendorf Catalog #022431021**
- 4% sucrose in 1X DPBS; kept at 4°C
-  DPBS (10X), no calcium, no magnesium **Thermo Fisher Scientific Catalog #14200075**
- 15mL conical tubes with 10% buffered formalin
- 15mL conical tubes with 4% paraformaldehyde (PFA) (fresh for 1 week, keep on ice)
-  Eppendorf Conical Tube 15mL **Merck MilliporeSigma (Sigma-Aldrich) Catalog #EP0030122151**



4% PFA Tissue Fixation of Ovaries

4d

- 1 Place each pre-weighed piece of tissue in either a 1.5mL Eppendorf tube or 1.5mL cryo tube.
- 2 Keep PFA tubes on ice. Transfer tissue specimen into a tube and refrigerate at 4 °C for 24:00:00 . 1d
- 3 After 24:00:00 , transfer tissue into 4% sucrose/PBS solution and incubate for 24:00:00 at 4 °C . 2d
- 4 After 24:00:00 , transfer tissue specimen to 70% EtOH (freshly made) and store at 4 °C until ready for paraffin embedding. 1d
- 5 Proceed with paraffin embedding of samples within 3 weeks.
- 6 Paraffin embedded tissue can now be used for H&E Pathology Review.

Formalin Tissue Fixation of Fallopian Tube, Uterine Body, and Cervix

2d

- 7 Place each pre-weighed piece of tissue in either a 1.5mL Eppendorf tube or 1.5mL cryo tube.
- 8 Place tissue specimen in a prepped and labeled tube. Keep at room temperature for 24:00:00 . 1d
- 9 After 24:00:00 , transfer tissue specimen to 70% EtOH (freshly made) and store at 4 °C until ready for paraffin embedding. 1d
- 10 Proceed with paraffin embedding of samples within 3 weeks.
- 11 Paraffin embedded tissue can now be used for H&E Pathology Review.