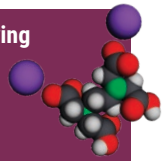
C U S T O M E R S T A L K , W E L I S T E N

HISTOLOGY TIPS & TRICKS

QUESTIONS & ANSWERS

What types of decalcifying agents can be used for downstream IHC/ISH analysis of specimens?



Decalcification of specimens like bone is performed after fixation to soften samples for sectioning while preserving morphology.

Thorough fixation is important prior to decalcification to prevent tissue damage due to the harsh nature of decalcifying agents.

Avoid strong acids such as hydrochloric and nitric acids. They decalcify quickly, but consequently impair downstream staining and destroy tissue architecture.

Weak acids like 10% formic acid are gentler and better preserve tissue architecture but are slower acting. Consider combining with 10% formalin or using a longer fixation period to avoid enzymatic degradation.

Chelating agents such as 14% EDTA are the gentlest decalcifying agents, however, are the slowest. Chelating agents preserve DNA and RNA due to its gentle nature.

Additional ways to optimize decalcification:

- Increase concentration of reagent
- Increase temperature
- Agitate sample
- Use 20:1 reagent-to-tissue for sufficient penetration and infiltration while ensuring total tissue access

How to avoid air bubbles in the tissue sections?

Ensure sections are properly fixed. Poor fixation can cause nuclear proteins to coagulate, particularly if exposed to heat that can cause a bubble effect.

High pH of fixatives can cause proteins to aggregate around liquid droplets within the tissue, causing bubbling. Check the pH prior to fixing.

Check there are no bubbles in the paraffin when heating. Agitation of the tissue while embedding may remove any bubbles from the cassette.

Remove air bubbles in the flotation bath to avoid bubbles becoming trapped underneath the section. This can later cause tissue detachment and distortion during downstream staining.

Ensure sufficient drying time for sectioned slides, without using excessive heat. Too much heat will cause rapid evaporation of water throughout the tissue, causing proteins to coagulate in a bubble effect.

How to overcome issues with cracks in tissue sample?

Overdehydration with alcohol after fixation can cause brittle tissue. Carefully optimize and monitor the time spent in alcohol solutions.

Excessive heat at the embedding center can cause cracks in the sample. Continuously monitor the temperature of both the hot plate and wax.

When sectioning tissue on the microtome, ensure the blade is secure, and free of contaminants as this can cause tears in the tissue when sectioning.

FFPE tissues cut well when cold, however avoid freezing blocks as this can cause the block to crack. Instead, chill on a cold wet surface.

Monitor and check the temperature of the flotation water bath. Excessive and prolonged exposure to heat can cause overexpansion of sections.

Drying the tissue on a hot plate without sufficient draining after mounting sections can cause cracks due to rapid evaporation. Ensure slides are adequately drained, and that the hot plate temperature is not set too high.

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