

Fixation Quick Card

Core controls: prompt fixation; tissue generally 3-5 mm thick; sufficient fixative volume; neutral buffered formalin near neutral pH; document time in/out for critical assays.

Mechanisms

Class	Examples	Key effect	Typical cautions
Crosslinking	Formaldehyde, glutaraldehyde	Preserves architecture through protein crosslinks	May mask epitopes; retrieval may be required.
Coagulant	Ethanol, methanol, acetone	Rapid protein precipitation; crisp nuclear detail	Shrinkage, brittleness, lipid extraction.
Oxidizing / heavy metal	Osmium, mercury-containing mixtures	Specialized morphology or lipid preservation	Safety, pigments, limited downstream compatibility.

Troubleshooting

Finding	Likely fixation contribution	First checks
Autolysis / weak nuclei	Delay or inadequate penetration	Cold ischemia, thickness, fixative volume, time.
Edge-center gradient	Specimen too thick	Re-gross if possible; document limitation.
Brown-black granular pigment	Acid formalin hematin	Confirm fixative/pH and validated pigment removal.
Weak IHC after long fixation	Excess crosslinking	Validate retrieval changes; do not over-retrieve blindly.

Always follow local SOPs and assay-specific pre-analytic requirements.