

# Fixation — Quick Card

Rapid reference for HT/HTL bench work: goals, preanalytics, fixatives, artifacts, QC, and IHC/ISH notes.

## Goals

- Arrest autolysis & putrefaction
- Stabilize morphology
- Preserve targets for downstream assays (H&E, specials, IHC/IF, ISH, enzymes)

## Rules of thumb

- 3–5 mm tissue thickness in cassette
- ~10:1 fixative:tissue volume
- NBF pH 7.0–7.2; RT for routine formalin
- Minimize cold ischemia ( $\leq 1$  h; immediate for biopsies)
- Record **time in** and **time out** of fixative

## Preanalytic checklist

- Specimen promptly into fixative (avoid dry time)
- Cassettes not overcrowded; agitation OK
- Fixative pH checked & documented
- Specimen thickness verified ( $\leq 5$  mm)
- Critical cases: note cold ischemia time

## Core fixatives — quick compare

Fixative	Type	Best use / Strengths	Watchouts
10% NBF	Crosslinking	Universal H&E; many IHC; gold standard morphology	Formalin pigment if acidic; epitope masking → retrieval
Glyoxal-based	Crosslinking	Routine histology; faster; less odor	Different retrieval vs NBF (validate)
Glutaraldehyde	Dialdehyde	EM; enzyme histochemistry; ultrastructure	Overhardening; autofluorescence; not for paraffin
Alcohols/Acetone	Coagulant	Cytology; frozen; enzyme activity; rapid	Shrinkage; brittleness; poor trichomes
Bouin's	Compound	Testis; GI mucosa; trichomes (CT contrast)	Yellow picrate; long washes
Zinc formalin	Additive	Improved nuclear detail; some IHC retention	Zinc waste handling; crystal precipitates

## Artifacts & removal

- **Formalin pigment (acid hematin):** brown/black granules after acidic/long fixation — remove with alcoholic picric acid or 10% ammonium hydroxide in 70% ethanol.
- **Mercury pigment** (legacy fixatives): remove with iodine → sodium thiosulfate.
- **Overfixation:** brittle tissue, micro chatter — soften block (ice/glycerol), trim thinner; tune retrieval/IHC.

## IHC/ISH notes

- Crosslinking masks epitopes → optimize retrieval (buffer pH/heat/enzymatic)
- For bone/ISH: prefer EDTA decalc over strong acids
- Validate when switching fixatives or retrieval; keep controls

## Safety

- Minimize formaldehyde exposure; use ventilation and PPE

- Handle picric acid wet; control inventory
- Treat mercury/zinc solutions as hazardous waste per policy

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