

# Standard Protocol for Formalin-Fixed Paraffin Embedded Tissue

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(From IHC World)

1. Fix tissues with 10% formalin or other fixatives for 24-48 hours at room temperature. Make sure you have enough fixative to cover tissues. Fixative volume should be 5-10times of tissue volume.
2. Trim fixed tissues into appropriate size and shape and place in embedding cassettes.
3. Process for paraffin embedding schedule as follow (total 16 hours):
  - 70% Ethanol, two changes, 1 hour each
  - 80% Ethanol, one change, 1 hour
  - 95% Ethanol, one change, 1 hour
  - 100% Ethanol, three changes, 1.5 hour each
  - Xylene or xylene substitute (i.e. Clear Rite 3), three changes, 1.5 hour each
  - Paraffin wax (58-60 °C), two changes, 2 hours each
  - Embedding tissues into paraffin blocks.
4. Trim paraffin blocks as necessary and cut at 3-10 um (5 um is commonly used).
5. Place paraffin ribbon in water bath at about 40-45 °C.
6. Mount sections onto slides.
7. Allow sections to air dry for 30 minutes and then bake in 45-50 °C oven overnight. **NEVER allow baking temperature go higher than 50 °C for sections thicker than 25um.** Otherwise sections may crack, especially 25-50 um thick sections, and result in sections falling off slides during staining.
8. The deparaffinized sections in 2-3 changes of xylene, 10 minutes each. Use three changes of xylene for sections thicker than 25 um.
9. Hydrate in 2 changes of 100% ethanol for 3 minutes each, 95% and 80% ethanol for 1 minute each. Then rinse in distilled water.