Standard Protocol for Formalin-Fixed Paraffin Embedded Tissue

(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 5-10 times 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 ºC), 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 ºC.

6. Mount sections onto slides.

7. Allow sections to air dry for 30 minutes and then bake in 45-50 ºC oven overnight. NEVER allow baking temperature go higher than 50 ºC 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 deparaffinize 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.