

### FIXATION: Samples for Paraffin

- **For most samples optimum fixation in 10% Neutral Buffered Formalin is achieved in 24 hours** with a fixation window of 8 hours to 48 hours.  
Under or over fixed tissue will not section well and may cause issues with future staining. Check the literature before starting your experiment to make for formalin fixation is compatible with future staining plans.
- **Samples should be no more than 3mm thick.**
- **Use a ratio of at least 1:10 tissue to fixative volume.**  
*If the ratio of fixative to sample is not at least 1:10 the fixative may not penetrate throughout the whole sample.*  
*Using a greater volume of fixative will not cause harm to the sample.*
- **After 24 hours the sample should be removed from fixative and moved to 70% ethanol.**  
To maintain the best morphology the core recommends moving through a series of graded alcohols (as outlined in the protocol below).  
If you are in a hurry you may move straight to 70% ethanol. When going straight into 70% EtOH use two changes of 70% EtOH to wash off the residual formalin and stop formalin fixation.
- **Please note that samples may be stored in 70% ethanol for up to one month.**  
After one month of storage in 70% EtOH you will may observe alcohol fixation artifacts in your tissue.

### BTBTC Core Optimal Fixation Protocol:

1. As tissue is collected rise in PBS or fixative and place in 10% Neutral Buffered Formalin
  - a. Completely cover sample using a ratio of 1:10 sample to fixative
  - b. If sample is placed into a cassette do not crush, trim sample small enough to fit or use a bigger cassette
  - c. Samples should be no more than 3mm thick to allow for penetration of fixative
2. **OPTIONAL:**  
After harvest move samples to 4 degree C refrigerator for 2 hours
  - a. Initial cold fixation can help improve future biomarker staining
3. After harvest or optional 2 hour cold fixation allow samples to fix at room temperature for up to 24 hours
  - a. Ideal fixation for most samples in 10% NBF is 24 hours total
  - b. The fixation window for most samples in 10% NBF is 8 to 48 hours
  - c. Smaller tissues such as needle biopsies may need less time while larger samples may tolerate more time in fixative
4. To stop formalin fixation and prevent tissue shrinkage move samples through a series of graded ethanol
  - a. 30% EtOH for 1 hour
  - b. 50% EtOH for 1 hour
  - c. 70% EtOH for at least 1 hour
5. Proceed to sample processing protocol

6. *NOTE:*

While it is recommended to process as soon as possible after collection samples may be stored in 70% EtOH for up to 1 month prior to processing.