

Research Pathology Services - Tissue Collection and Fixation Guidelines

-We generally fix in 10% neutral buffered formalin (NBF) for at least 48hrs. It is good for basic histology, most special stains, and can also be used for many IHC protocols.

-For most purposes, you can leave the tissue in formalin until processing. (If your protocol calls for transfer to 70% EtOH, that is ok. Just note that bloody tissues such as placenta, spleen, liver, etc. tend to dry out with long term storage in ethanol.) *For large tissues that are not pre-trimmed down for various reasons, such as orientation-specific trimming, we highly recommend leaving in formalin until submission!!!*

-Tissue that is dropped fixed (not perfused first) should be no larger than 3-4 mm x 3-4mm.

-If it is larger, it should be trimmed down to this size prior to fixation, or at least within 24 hrs after beginning fixation, if you have delicate structures that will trim better after firming up a bit.

-Make sure to use a minimum of 10-20 volumes of formalin (or other fix) to volume of tissue.

-For formalin fixed tissues, fix at room temperature (do not put in the refrigerator, this slows penetration of 10% NBF).

-Flat bottom containers are best, but it is ok to use 15 or 50 ml conical tubes as long as you can fit 10-20 volumes of formalin in and the tissue is **freely floating** in the formalin. **If you use conical tubes, place them on their side or upside down during the first 48 hrs.** You do not want the tissue sitting in the bottom of the conical. It will not fix well and will take the shape of the tube.

-It's best if you can **keep the container gently agitating** on a shaker table or rocking table for at least the first 24 hrs. If you don't have access to one, you should swirl the container of tissue/cassettes several times in the first 24 hours. Formalin gets "used up" locally, so it helps to agitate to continuously expose the tissue to "fresher" formalin.

-In addition, **for large and/or bloody samples, change out the formalin the first 24 hrs.** You can also drop off the tissues to us in formalin the day of collection, and we will do the above steps for you. If you have staggered collection dates, make sure you perform the above for each set of tissue collections.

-It is permissible to fix tissue in cassettes, provided there is ample room so that the tissue is not touching both top and bottom of the cassette when it is closed. **If your sample is more than 3mm thick, you should not use standard cassettes.** When thick tissue is squeezed in a cassette, it not only impedes penetration of the formalin, slowing fixation, but also creates compression artifact. If you think your tissue will be too large for a standard cassette, we can supply you with deeper cassettes for your study.

