Sample Preparation and Shipping Instructions

Preparation:

The fixative used to prepare histopathology and IHC samples is determined by the specific experimental goals, tissue type, and downstream applications.
Below are some commonly used fixatives and how to prepare them:

1. **Formalin**: Formalin is the most used fixative for histopathology and Immunohistochemistry (IHC) samples.

To prepare fresh 10% neutral buffered formalin (also referred to as fresh neutral buffered 4% paraformaldehyde), dissolve 40g of formaldehyde in 900 ml of distilled water and add 100 ml of 10X phosphate-buffered saline (PBS). Adjust the pH to 7.4 using sodium hydroxide (NaOH) or hydrochloric acid (HCl) and bring the volume to 1 L with distilled water. Most commercial purchased 10% formalin contains 10-15 % methanol as a preservative.

2. **Ethanol**: Ethanol is a good fixative for preserving lipids and cellular membranes for Histopathology but not for IHC samples.

To prepare 95% ethanol fixative, mix 95 ml of absolute ethanol and 5 ml of distilled water.

3. **Methanol**: Methanol is a commonly used fixative for frozen sections and immunofluorescence and IHC assays.

To prepare methanol fixative, use absolute methanol.

4. **Bouin's fixative**: Bouin's fixative is used for preserving glycogen and elastic fibers. For Histopathology samples only

To prepare Bouin's fixative, mix 75 ml of saturated picric acid, 25 ml of 37% formaldehyde, and 5 ml of glacial acetic acid.

5. **Acetone**: Acetone is commonly used for preserving lipids and cellular membranes used for frozen and cell cultures for IHC and immunofluorescence assays.
6.

To prepare acetone fixative, use absolute acetone.
There are a number of specific fixation solutions for particular tissue such as Davidson Fixative for eyes only.

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Fixation:

There are different methods followed for various situations, below explained are two common methods of fixatives.

Types of fixatives:

Perfusion fixation is a method of preserving biological tissues for further study or experimentation. The fixative solution is injected directly into the bloodstream by cardiac or vena cava puncture, allowing the fixative to permeate the tissues and preserve their structure and molecular content.

Below explains the general steps taken to perform Perfusion fixation:

1. Anesthesia: The animal or organism is anesthetized to minimize pain and discomfort during the procedure.
2. Cannulation: A cannula (a thin, flexible tube) is inserted into a major blood vessel, usually into left ventricle of heart and also aorta or the vena cava.
3. Perfusion: A fixative solution, such as formalin, formaldehyde or paraformaldehyde, is pumped through the cannula into the bloodstream. The fixative is typically delivered at a slow and steady rate, usually between 5-10 ml per minute, to ensure uniform penetration throughout the tissues.
4. Post-fixation: After perfusion, the tissues are usually left in the fixative solution for an additional period of time to ensure complete fixation.
5. Tissue preparation: The fixed tissues are then typically dissected or sectioned for further analysis or experimentation.

It should be noted that the specifics of perfusion fixation can differ depending on the type of tissue being studied and the desired experimental outcome. The general steps outlined above, however, provide a basic overview of the technique.

Immersion Fixation: it involves the use of fixative solutions. Since the solution should completely diffuse through the sample, the size and density of the sample should be a major consideration. For best results, the sample should be immersed in the appropriate fixative at a minimum volume of 20 times greater than the sample.

The general steps involved in immersion fixation are as follows:

1. Dissection: The tissues of interest are carefully dissected and isolated from the rest of the organism or sample.
2. Fixative preparation: A fixative solution, such as formaldehyde, is prepared according to the desired concentration and volume.
3. Immersion: The dissected tissues are then placed directly into the fixative solution and left to soak for a set period of time. The exact duration of immersion will depend on the type of tissue being studied and the desired level of fixation.
4. Post-fixation: After immersion, the tissues are typically left in the fixative solution for an additional period of time to ensure complete fixation.

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5. Tissue preparation: The fixed tissues are then typically dissected or sectioned for further analysis or experimentation.

It is important to note that immersion fixation can result in uneven penetration of the fixative solution, resulting in incomplete fixation or tissue structure distortion. Some researchers may use agitation or other techniques to ensure uniform distribution of the fixative solution to mitigate these effects. Furthermore, the specifics of immersion fixation can vary depending on the type of tissue under investigation and the desired experimental outcome. The general steps outlined above, however, provide a basic overview of the technique.

Promptness & Volume of Fixative:

Prompt fixation is critical to preventing tissue sample degradation caused by autolysis and other factors. Tissues should be fixed as soon as possible after being collected, ideally within minutes. The longer the time between tissue collection and fixation, the greater the risk of tissue damage and degradation, which can jeopardise histopathological analysis's accuracy and reliability.

The amount of fixative needed for histopathology samples is determined by the size and type of tissue being studied, as well as the desired level of fixation. To ensure adequate penetration of the fixative solution, the volume of fixative should be at **least 20 times the volume of the tissue to be fixed**. **Perfusion fixation may be required for larger tissues**, such as organs or whole bodies, to ensure complete fixation.

It is also critical to select the correct fixative solution for the tissue sample.
Different fixatives have different properties and may be better suited to specific tissues or analyses. Although formalin is a widely used fixative that is effective for a wide variety of tissues, other fixatives such as **ethanol** or **Bouin's** solution may be more appropriate for specific tissue types or research questions.

To summarise, both prompt fixation and an adequate volume of fixative are required for high-quality histopathology samples.
The specific requirements for fixative promptness and volume will vary depending on the tissue type and research question, but in general, tissues should be fixed as soon as possible after collection, and the volume of fixative should be at least **20 times** the volume of the tissue being fixed.

Length of Fixations:

The amount of fixation time required for histopathology and immunohistochemistry (IHC) will vary depending on the type of tissue being studied and the specific experimental goals. Fixation times can vary from a few hours to overnight or longer.

The length of fixation should be sufficient for **histopathology** to ensure optimal preservation of tissue morphology and cellular structures. Formalin fixation is commonly used in histopathology, with fixation times ranging from several hours to overnight depending on the size and type of tissue being studied.

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Over-fixation can cause tissue hardening and antigenic site masking, whereas under-fixation can result in poor cellular structure preservation.

For IHC, the fixation time should be long enough to preserve antigenicity while also preserving tissue morphology. Over-fixation can result in antigenicity loss, while under-fixation can result in poor tissue morphology preservation. Shorter fixation times are generally preferred for IHC to preserve antigenicity. Fixation times of 6-24 hours (maximum 48 hrs) are commonly used for IHC using formalin-fixed paraffin-embedded (FFPE) tissue to preserve both tissue morphology and antigenicity.
On the other hand, it is important to note that the length of fixation may also be affected by the specific antibody used for IHC, as some antibodies may necessitate longer fixation times to maintain antigenicity.

Furthermore, the type of fixative used may influence fixation times. Ethanol-based fixatives, for example, require shorter fixation times than formalin-based fixatives.

Dehydration for Paraffin Embedding:

After fixation, remove the tissues from the NBF solution and rinse them in distilled water to remove excess fixative.

Place the tissues in a container filled with 70% ethanol solution to dehydrate the tissue.
Label the container with the appropriate sample identification information.
Allow the tissues to sit in the 70% ethanol solution for at least 24 hours or until they are fully dehydrated.

Following fixation samples for IHC and Immunofluorescence assays:

After fixation, remove the tissues from the NBF solution and rinse them in Neutral phosphate buffered saline (PBS, pH7.4) to remove excess fixative.

Label the container with the appropriate sample identification information.
Store tissues in PBS at 4^0 C

This tissue can be paraffin embedded or frozen (cryosection) for IHC or Immunofluorescence assays.

Shipping:

Histopathology and IHC samples must be shipped and packaged with special care to ensure that they are not damaged during transit and arrive in usable condition.
Below is a general approach to shipping and packaging these kinds of samples:

1. Samples can be shipped in formalin for all subsequent downstream procedures. Samples for frozen cyrosectioning for IHC and immunofluorescence assay can be shipped in PBS instead of formalin depending on timelines. Also, samples to be paraffin embedded can be shipped in 70% Ethanol.

2. Use leak-proof and sturdy packaging materials such as plastic bags, plastic containers, or cardboard boxes. The packaging materials should be resistant to temperature and humidity changes.
3. Select a reliable courier or shipping service that can deliver the samples quickly and safely. (Most labs have their preferred method of delivery and courier service)
4. Label the samples with all relevant information, including the sample name, the date of collection, the type of sample, and the test requested.
5. For liquid samples, add absorbent material such as paper towels or cotton to prevent leaks. Could use paraffin wax for the lids of sample containers to ensure no leaks occur.
6. For samples that require refrigeration, add cold packs to the packaging to maintain a constant temperature during transit.
7. Place the labeled and appropriately packaged samples in a secondary container, such as a plastic bag, Styrofoam cooler, or a plastic container, to further protect them during transit.
8. Add cushioning material such as bubble wrap, packing peanuts, or shredded paper to fill any empty spaces and prevent movement of the samples during transit.
9. Seal the primary and secondary packaging securely with tape or plastic seals.
10. Ship the package using a reliable courier or shipping service and track the shipment to ensure that it reaches its destination in a timely manner.
11. Ship samples early in the week to avoid delays and hold ups over the weekend.

Overall, the type of sample, the required temperature conditions, and the shipping method must all be carefully considered when shipping and packaging histopathology and IHC samples. Proper sample labelling, packaging, and cushioning can help ensure that the samples arrive in good condition for further analysis.

If you have further questions regarding preparing or shipment of tissue, contact info@waxitinc.com or call 604-822-1591