hywax
green tech

Histology Waxes

Personal Care

Product Information

Origin

Histology waxes are produced by subjecting paraffin waxes to highly sophisticated refining processes, including unique crystallization and de-oiling technologies.

Purity

Hywax GmbH utilizes high-pressure hydrogenation, the most cutting-edge purification process, to produce histology waxes without any impurities. This production method guarantees a highly purified product that is ideal for use in histological laboratories. A dedicated product line and a multistage filter system ensure the highest-quality pastillation of the product to match the most exacting requirements.

Chemical structure

Histology waxes are essentially complex multicomponent combinations of saturated hydrocarbons, with medium-length hydrocarbon chains. The purity and inert properties of the waxes guarantee the perfect handling of all tissue samples.

Packaging

Neutral bags, 20 kgs net

A dedicated and full covered product line and an optimized filter system ensure highest quality pastillation of these products.

General Properties of our Histology Waxes

- Highest purity
- Fast and straightforward embedding process
- Good compatibility with the infiltrated sample
- Easy positioning of the tissue sample
- No cracking
- Excellent microtome sectioning results and ribbons
- Gentle on microtome blades
- Highly soluble in xylene

Histology Wax 0587 (54/56) - infiltration and embedding

- Exceptional flexibility
- Easy demolding
- Excellent suppression of crystallisation
- Enhanced with special additive
- Fast tissue penetration
- Lucent wax

Histology Wax 0599 (58/60) - embedding

- Exceptional flexibility
- Easy demolding
- Excellent suppression of crystallisation
- Enhanced with additive
- Increased melting point: particularly suitable for use in high-temperature environments

Histology Wax 0600 (56/58) - embedding

- Exceptional flexibility
- Easy demolding
- Excellent suppression of crystallization
- Enhanced with special polymer-additive

Histology Wax 0700 (54/56) - infiltration and embedding

- Exceptional flexibility
- Enhanced with very special additive
- Excellent suppression of crystallization
- Easy demolding
- Fast tissue penetration

Features and Processing

Histology waxes

Product	Congealing Point °C	Application Temperature °C	Water bath Temperature °C	Cooling Temperature °C
HISTOLOGY WAX 0587	54-56	60-62	40-45	-2 to -6
HISTOLOGY WAX 0599	58-60	65	45-55	-2 to -6
HISTOLOGY WAX 0600	56-58	62-65	45-50	-2 to -6
HISTOLOGY WAX 0700	54-56	60-62	40-45	-2 to -6

FAQ - Frequently Asked Questions

FAQs

Question	Answer
How long should the tissue samples be kept in the fixation bath?	24 to 26 hours, minimum 24 hours.
What happens if I shorten the time for fixation?	Reducing the time for fixation can lead to considerable problems with producing the microtome sections
How long should dehydration last?	For optimum results, no tissue samples should be subjected to dehydration for periods longer than 12 hours (or the time recommended by the manufacturer). It depends on the thickness of the tissue; very small tissue can be dehydrated in a couple of hours. Super-mega sections need much longer for dehydration.
Why should the paraffin wax for infiltration be changed regularly?	Xylene can have a negative effect on the infiltration of the new sample. Xylene traces in the sample can also lead to cracks and holes in the tissue sample.
Why should tissue samples be embedded at warm temperatures?	The tissue sample and embedding frames should be kept in a container heated to approx. 60 °C to avoid any interference coming from a difference in the temperatures of the tissue, the frames and the paraffin wax used for embedding. The metal mould is filled with the liquid paraffin wax to the first rim. Tissue samples should then be positioned quickly in the already-settling wax. The forceps used for handling the sample should also be (pre-) heated to prevent the tissue sample or paraffin wax from sticking to the instrument. All of these precautions are necessary to ensure a good bond between the infiltrated sample and the embedding wax.

Question	Answer
Why should the temperature of the paraffin not alter during embedding? (See table features and processing)	This is the ideal temperature for embedding the tissue sample in the paraffin wax. The paraffin wax settles optimally at this temperature for a better connection between the wax and the infiltrated sample.
Is there any advantage in choosing a very low temperature for the cooling plate to speed up the cooling process?	There is no advantage! A large difference in temperatures can lead to cracking of the paraffin block, which would affect the tissue sample. After regular cooling, the paraffin and the tissue sample should have reached the desired temperature. The paraffin wax shrinks during cooling, which allows the block to be removed from the mould and the blocks to be cut in the microtome. Ten minutes are recommended as the optimum cooling period (at a temperature of -2 °C to -6 °C) for paraffin blocks of approx. 7 g.

Guideline for histological application

Fixing the tissue samples

There are various fixatives, all of which have their own particular characteristics. A widely used fixative is that in aqueous formaldehyde solution. The advantage of formaldehyde is that the tissue can be kept in it for a long time without significant changes in structure.
For the commonly used H/E (hematoxylin/eosin) staining, it is an excellent solution. Fixation stops decomposition. For special tissue samples, other fixatives are used, e.g. ethanol.
The tissue pieces should not be too large. The larger the piece, the greater the risk that autolysis (cell decomposition) of the tissue will begin. In exceptions, it may be useful for larger objects to be warm pre-fixed. For best results, the tissue should then preferably be further fixed cold. In this case, autolysis progresses more slowly, but fixation also takes longer. Small pieces (approx. 5 x 5 x 5 mm) need at least 24h, 26h is better. Mistakes made here (e.g. shortening the fixation time) are irreversible and lead to massive problems later when making the sections in the microtome.

Dehydration

After the fixative has been rinsed off with water, the actual dehydration can begin. The aim is to slowly remove the water from the tissue. First dehydration is done with an ascending alcohol series (ethanol) and later the ethanol is replaced by paraffin. In some laboratories, dehydration and infiltration with paraffin are carried out in an automatic machine.
The following steps are carried out in this process:

Dehydration with:
- 50% ethanol
- 70% ethanol
- 85% ethanol
- 95% ethanol
- 100% ethanol / isopropanol

Exchange ethanol for xylene, then for paraffin:
- Xylene
- Xylene/paraffin
- Paraffin

The 100% ethanol in the last step of dehydration can also be replaced by 100% isopropanol, because pure anhydrous ethanol quickly attracts humidity. The use of Acetone is not recommended in normal histology because of the drastic shrinkage of the tissue.

The literature states: "For best results, small tissue samples (5 x 5 x 5 mm) should remain in each bath for 12 hours, while larger pieces should remain in each bath for 24 hours".

Typically, this process takes less time in the vending machine, but should not be shortened contrary to the manufacturer's recommendations in order to save time. This process should take enough time to achieve good results in the microtome at the end. Mistakes made here are irreversible.

After 100% isopropanol, the xylene step follows. In this step, the tissue becomes slightly translucent. It is important not to leave the tissue in the xylene longer than necessary, otherwise it will become too hard.

Samples of liver or spleen, for example, can become so hard that they are no longer cut or are difficult to cut. At the end of dehydration, the water from the tissue is replaced by xylene.

In the last step, the xylene is again completely removed and replaced by paraffin.
For this, a warm mixture of xylene and paraffin (1:1) is used in the first step. The next step is infiltration with fresh and clean paraffin. The xylene must be completely replaced by paraffin, a xylene smell is not acceptable! The dwell time in liquid paraffin at low temperatures (60°C) has in principle no maximum, it does not harm the tissue. However, we do not recommend storing in liquid paraffin for longer than 48h. However, if residues of xylene remain in the fabric, it is more difficult to cut and leads to problems such as tears or holes in the fabric.
After this last step, the tissue is embedded in the paraffin.
The temperature of the paraffin should not exceed 64°C here either.

Embedding in paraffin

When using standard embedding cassettes, these fit exactly into the mould. The mould is made of stainless steel so that it can be reused.

For embedding, a different paraffin (mixed with special additives) is usually used than for infiltration. These paraffins are specially formulated for this application and give the best results when solidifying, embedding and cutting in the microtome.

The tissue should be kept ready with the embedding cassettes in a warm container of approx. 60°C to prevent difficulties due to temperature differences between the tissue, cassettes and embedding paraffin.

The metal mould is filled with the liquid paraffin up to the first rim. The tissue must then be positioned quickly in the already solidifying paraffin. The forceps must be well warmed up or heated beforehand, otherwise the tissue sample or the paraffin will stick to them.

The embedding machines usually used today have a small cooling and heating plate to simplify positioning and a storage vessel with paraffin at a temperature of 60-64°C (daily requirement). The paraffin is added via a dosing device.
Then the PE cassette is placed with the bottom down and the mould is completely filled with paraffin. The embedding cassette must be completely surrounded by paraffin in the mould without overflowing. The paraffin must never be warmer than 64°C to prevent cracks from forming when the mould is subsequently cooled on the cooling plate.

The mould is usually placed on a cooling plate so that the paraffin wax can cool and solidify. However, an extremely cold cooling plate and too long a storage time can lead to cracks in the paraffin block under certain circumstances. This later has a negative effect on the behavior when cutting in the microtome.

The paraffin shrinks somewhat when cooling on the cooling plate, which can be visible on the surface through a small indentation. This is a normal and desired property of the paraffin so that it can be easily removed from the mould later.
The numbered blocks can now be cut in the microtome and then archived. The paraffin blocks, if everything has been done correctly, can be stored for many years according to local specifications.

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