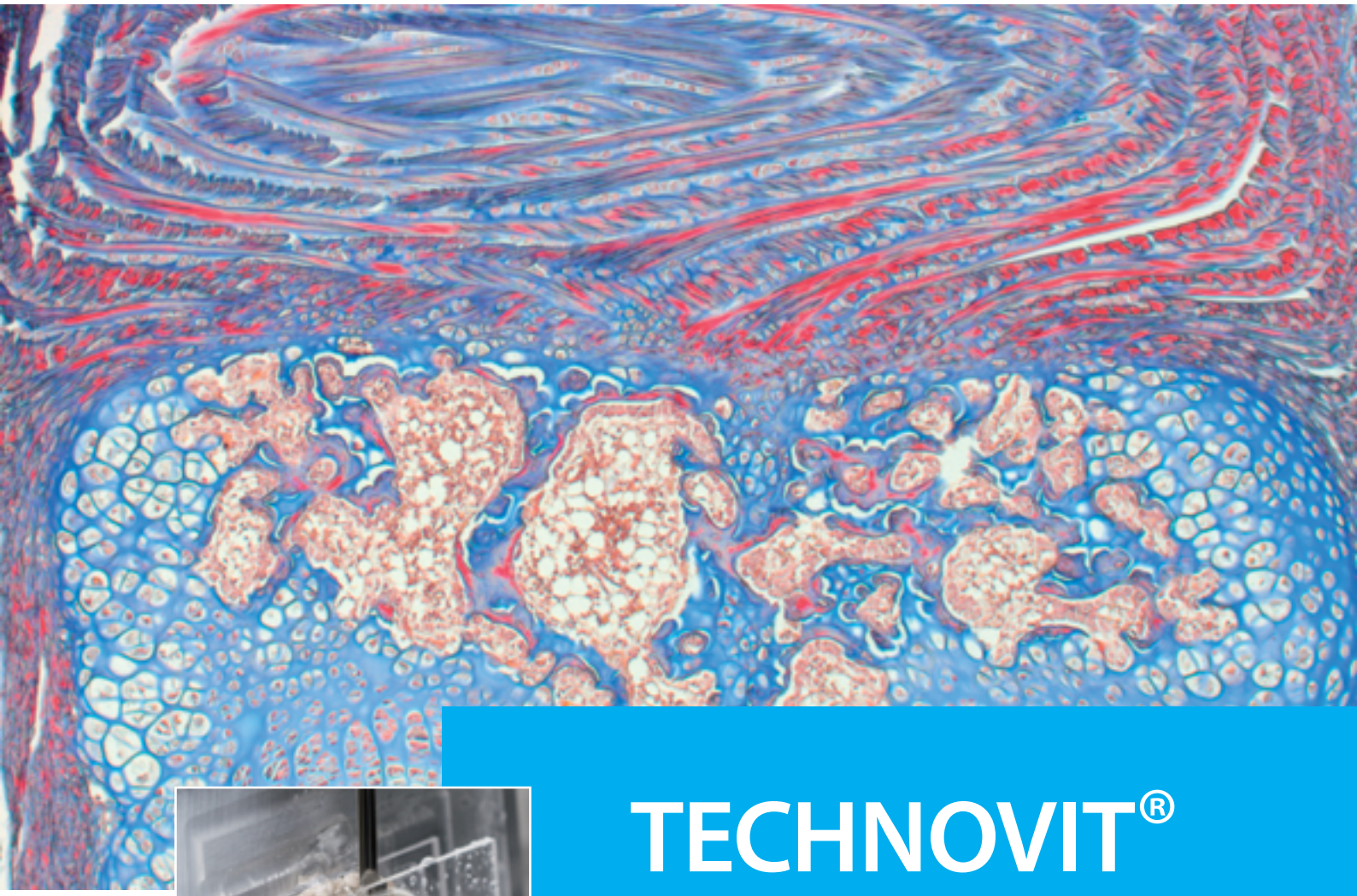
MORPHISTO
Laboratory chemicals & Histology service



TECHNOVIT®

Polymerisation Systems for Histological Applications

www.morphisto.de

KULZER
MITSUI CHEMICALS GROUP

Technovit®

Polymerisation Systems for Histotechnology

Modern research and diagnostics increasingly require the production of histological sections of hard materials such as nails, cartilage, bone, teeth and implants. The Technovit® resins from Kulzer GmbH have proven themselves in practice for decades. They are used for diagnostic and research purposes in all areas of biology and medicine as well as materials science. The resins have excellent infiltration properties and can be cut with conventional microtomes or sawed, ground and milled with special cutting-grinding systems (e.g. from EXAKT).

The polymerisation systems meet important requirements, such as low temperature embedding, thin and semi thin section technology as well as optimal cutting and grinding properties. The sections can be stretched easily and the stained specimens show excellent morphology under the light microscope.

With the Histo-Technik von Kulzer GmbH the scientific and economic conditions for histological examinations of tissues are considerably improved.

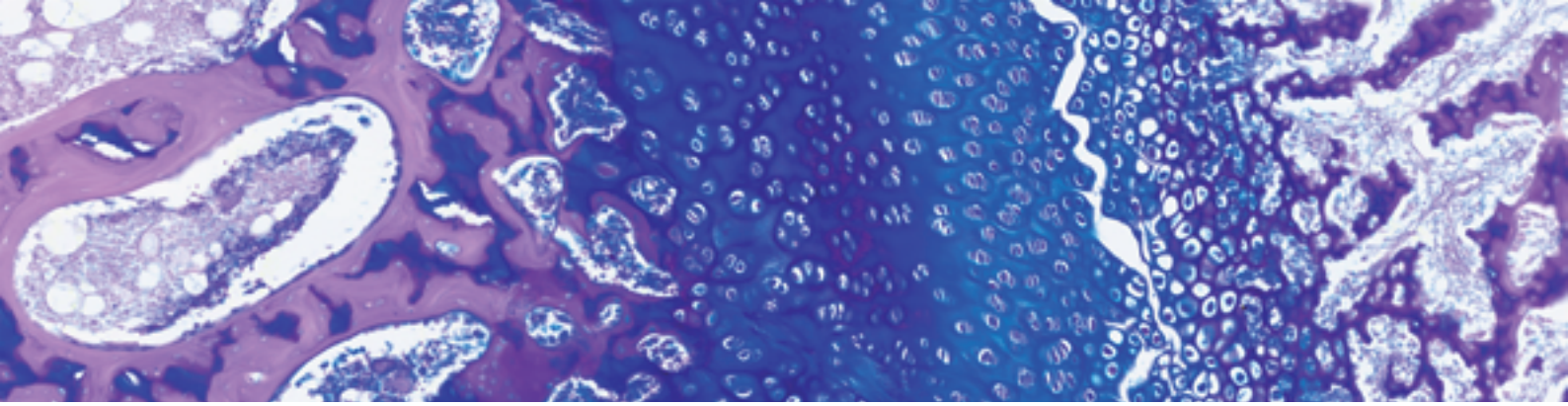
- **Easy handling, as all components are coordinated with each other.**
- **Due to the special material properties, the standard staining methods, enzyme and immunohistochemistry, including in-situ hybridization, used in histological laboratories can be performed.**

Why resin?

In contrast to all other embedding materials used in light microscopy for histological techniques, constant thin and semi-thin sections can be made after resin embedding. Morphological details are excellently preserved. In undecalcified samples embedded in resin, the mineralized and cellular structures can be better determined. Both the mineral matrix and the cartilaginous and ligamentous tissues are very well preserved.

The results of enzymatic, immunohistochemical investigations and also of in-situ hybridisation show a more sensitive and specific activity, as all Technovit resins cure at low temperatures and even sub-zero temperatures due to the special composition of the polymerisation systems.

Content



Technovit® 7100

Sections for histological and industrial use

Technovit 7100 is a plastic embedding system based on HEMA (2-hydroxyethyl methacrylate, or GMA = glycol methacrylate). The hydrophilic resin is used in medicine, botany, zoology and in industry for embedding tissues for light microscopic examinations. The sections can be used for histological staining and enzyme detection.

Technovit 7100 polymerises transparently. The blocks can be used to produce uniformly thin sections on the rotation microtome. It is neither necessary nor possible to remove the resin from the block and the section.

The advantages at a glance

- easy handling.
- Reproducibility and reliability of the embedding through constant, documented quality controls of the individual components.
- Low polymerisation temperature due to Teflon embedding forms.
- homogenous hardness of the block, thus ensuring even, very thin sections.
- Low shrinkage artifacts, therefore excellent tissue morphology.
- Besides routine staining also enzyme detection is possible.
- Less toxic due to barbituric acid catalyst.
- Polymerisation at room temperature (20 °C)
- No airtight seal required during curing.
- No decalcification is required for haematological iliac crest biopsies.

Material properties

The chemical polymerisation of Technovit 7100 is initiated by means of a barbituric acid derivative in combination with chloride ions and benzoyl peroxide. The catalyst system does not contain any aromatic amines compared to conventional systems.

Application

Apply Technovit 7100 according to the step by step instructions. Place the fixed and dehydrated samples first in the pre-infiltration solution and then in the infiltration solution. Vacuum intervals and/or agitation during the individual steps accelerate the embedding process. Samples for industrial applications are added directly to the infiltration solution or cured without pretreatment.

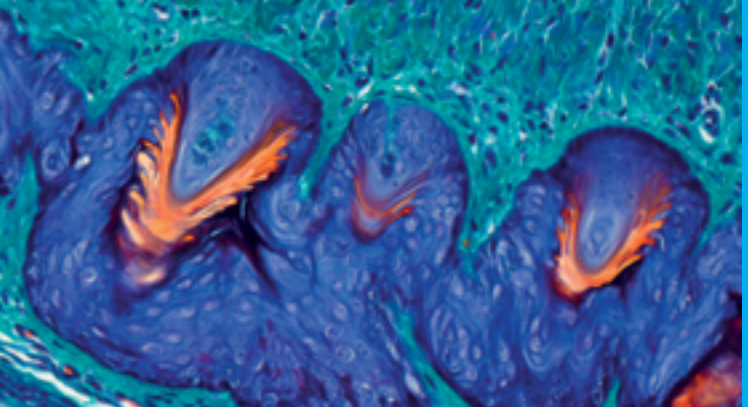
Polymerisation

Prepare the polymerisation mixture according to the instructions, fill it into the embedding moulds and immediately orient the infiltrated samples therein. Polymerisation is carried out at the following temperatures:

Polymerisation temperature in:

- Histoform S 32 °C – 38 °C
- Histoform Q 40 °C – 45 °C

After curing (approx. 1½ h) block up with Histobloc and Technovit 3040 (see also page 15).



Technovit 7100 is used in many areas: organic compounds, plastic materials, Films & Paper, Fibres and many more...

Further application areas

When it comes to making cuts of embedded material Technovit 7100 is THE material of choice. Originally developed for histology, Technovit 7100 has also proven itself in industry for years due to its universal application options.

Application areas are, for example, embeddings and sections of:

- Plastic materials
- Films
- Paper
- Textiles
- organic compounds
- Fibres

Material properties

In cases where samples are cut and examined under the light microscope, embedding with Technovit 7100 allows sections in the µm range. With the Technovit Histoblade disposable knife, which is specially adapted to this system, the samples can be sectioned cost-effectively and in high quality.
The preparation of these samples differs only slightly from that of medical samples. In many cases, it is not necessary to place them in the preparation solution, as is the case with films and plastic materials, for example.

Notes on embedding non-medical tissue samples

- For paper and textiles, infiltration in the preparation solution under vacuum is recommended.
- Biological preparations are treated like medical samples (fixed, drained and then infiltrated).
- Materials that are less temperature sensitive can also be embedded in larger moulds than the Teflon embedding forms S and Q.

Product data

Catalog no.	Description	Content
12227.K0500	Technovit 7100 Combipack	500 ml Basic solution 5 x 1 g Hardener 1 1 x 40 ml Hardener 2

Technical data	
Colour	transparent
density = specific weight g/cm3 (DIN 53479)	1,07
Refractive index monomer polymer	 1,4540 1,5050
Storage temperature	max. 25 °C
Shelf life	2 years

The processing

Depending on the nature of the industrial sample, e.g. non-porous materials such as foils etc., steps 1 - 4 (fixation to infiltration) can be omitted. The polymerisation can be started directly.

Fixation

Fix the tissue as required and use an adequate post-treatment.

Dehydration

The dehydration is carried out in ethanol or acetone, a stepwise dehydration with HEMA in buffer is also possible (+4 °C).
Alternating vacuum and agitation are helpful for better penetration during the embedding process. Intermedium is not necessary before pre-infiltration.

Pre-infiltration

Prepare the last alcohol solution of the dehydration series in equal parts with base solution Technovit 7100 and incubate for approx. 2 h at room temperature (20 °C) after addition of the samples (e.g. in 50 ml 96 % ethanol : 50 ml base solution Technovit 7100).

Infiltration

- Basic solution Technovit 7100 100 ml
- + Technovit 7100 Hardener 1 1 g (1 Bag)

Dissolve in a clean (detergent-free) glass or polyethylene container for approx. 10 min. The infiltration solution is sealed and stable at 4 °C for a maximum of 4 weeks.
Depending on the size, infiltrate the samples in a sufficiently large volume of the infiltration solution for up to 24 h (room temperature) or longer at 4 °C. A short vacuum (water jet pump) and agitation are helpful.

Polymerisation

Preparation of the polymerisation solution (disposable containers):

- Infiltration medium (unused) 15 ml
- + Technovit 7100 Hardener 2 1 ml

mix for about 3 - 5 minutes.
Use commercially available pipettes aids for this purpose!

Embedding in Teflon moulds

Fill half of the embedding moulds of the Histoform with the polymerisation solution (disposable pipette), orient the prepared sample in it and fill the form **(only the mould, not the entire recess).** Polymerisation is carried out either completely (2 h) at room temperature or 1 h at room temperature and then 1 h at 37 °C in an oven.

During polymerisation the plastic heats up. Certain temperature ranges must not be exceeded in order to prevent the block from becoming brittle and then no longer be sectioned. The following table shows the dependence of the maximum polymerisation temperature on the ambient temperature.

Ambient temp.	Histoform S	Histoform Q
Room temp. ca. 20 °C	32 °C	37 °C
Refrigerator 4 °C	16 °C	23 °C
Refrigerator on ice 0 °C	10 °C	18 °C

The slightly sticky surface (inhibition layer) can be wiped off with a lint-free disposable cloth. **Too high humidity encourages a stronger inhibition layer!**

Preparation of solutions in an overview

Solution	Ethanol	Basic solution Technovit 7100	Hardener 1 Technovit 7100	Infiltration-solution	Hardener 2 Technovit 7100	Application-temperature
Pre-infiltration	1 part	1 part				Room temperature
Infiltration		100 ml	1 g (1 Bag)			Room temp. / 4°C
Polymerisation S Q				15 ml 30 ml	1 ml 1,5 ml	Room temp. / 32°C Room temp. / 37°C

Blocking out, further processing, archiving

Technovit 3040

To release the polymerised blocks from the Teflon moulds, the Histobloc carrier parts are glued to the polymerised blocks with another Technovit resin and finally pulled out of the mould.

Technovit 3040 is a fast-curing 2-component resin based on methyl methacrylate (MMA) which, due to its chemical composition, forms a stable, permanent bond with the cured block Technovit 7100 (as well as Technovit 8100 or Technovit 9100).

Histobloc – Support parts

The Histobloc carriers are adaptable for the Histoform S and Q or also for the Histoform N and can be clamped firmly in all microtomes with universal holders.

Procedure

After complete polymerisation, the Histobloc carrier part is placed in the recess provided in the Histoform S/Q Teflon embedding mould.

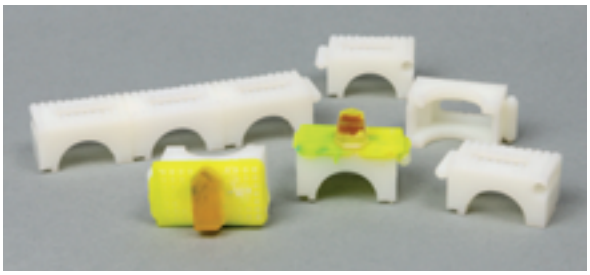
Mix Technovit 3040 as viscous as possible (mixing ratio around 3 : 1 / powder : liquid) and pour the mixture into the recess of the Histobloc.

Depending on room temperature, Technovit 3040 is cured in 5 - 10 minutes and the Histobloc is firmly attached to the sample.

The sample can now be removed from the mould and is ready for cutting. Blocking with the very hard Technovit 3040 stabilises the elastic Technovit 7100 or Technovit 8100 in such a way that even the thinnest cuts are possible.

Further processing

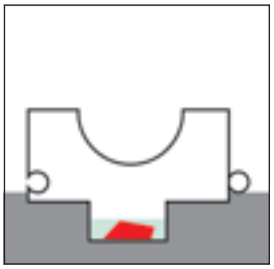
Clamp the blocks firmly in the jaw clamping device on the rotary microtome. To cut the blocks, use a Histoblade (in combination with the Kulzer knife holder) or carbide knife (note the knife angle). Remove the cut dry with tweezers and apply to a water bath for stretching (aqua dest.). Apply to clean, grease-free, coated or uncoated microscopic slides. Allow to dry at least 15 minutes or overnight at 60 °C before staining. Transfer the non-deplasticized sections directly into the staining solution. A 5 µm section must be stained longer than a 1 µm section to achieve the desired colour intensity.

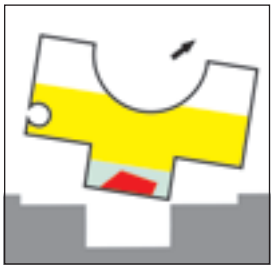


Storage and archiving of the samples

The Histoblocs can be plugged together for better archiving. This enables consecutive numbering and space-saving storage.

For histochemical and immunohistological investigations, it is recommended to store the blocks in PE bags at 4 °C or longer even at -20 °C.





Cutting on the microtome

Technovit 7100 samples can be sectioned on commercially available microtomes in thicknesses down to 1 µm. D-ground blades in a carbide design are used for this purpose. Normal D-knives can be used, but do not provide optimum results.
Disposable carbide knives are only suitable if they are designed as a version especially for cutting resins.

The disposable histoblade from Kulzer is such a carbide blade which is especially suitable for cutting tissue samples embedded in HEMA (2-hydroxyethyl methacrylate) as well as materials in industrial applications up to 1 µm cutting thickness. Together with the stable blade holder, this provides a low-cost alternative to other blades that can be used in all commercially available microtomes.

For this purpose there are different blade holder:

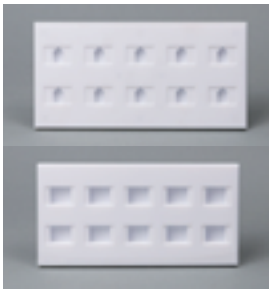
- Knife holder „NR" or „SL" for Microm/Thermo-Microtomes; Knife angle adjustment 9°
- Knife holder „N" for Reichert-Jung/Leica-Microtomes; Knife angle adjustment 0°

The advantages:

- Heavy, stable design
- Easy handling
- Good sectioning results up to 1 µm cutting thickness
- Optimized cutting qualityt
- Improved service life

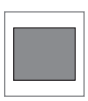
Dimensions:
Histoblade: 60 x 19 x 1 mm, 2 Holes à 4 mm
Knife holder: 170 x 34 x 10 mm

Accessories and supplements



Histoforms S & Q:
Special embedding moulds made of Teflon with stainless steel bottom for heat dissipation with 10 wells.

Histoform S
Dimensions: W x H x D:
ca. 10 x 16 x 6,5 mm.



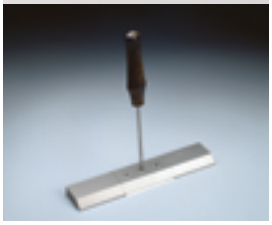
Histoform Q
Dimensions: W x H x D:
ca. 20 x 16 x 10 mm.

Histobloc - Carriers
The Histobloc carriers are adaptable for the histoform S + Q, as well as for the histoform N or N+ (Technovit 9100).

Mixing spatula
Wood spatula for mixing Technovit 3040.

Technovit 3040
Fast curing 2-component resin for blocking and fixing of cured samples on Histoblocs.

Mixing cup
Paper cup for mixing Technovit 3040.



Histoblade und Knife holder
Histoblade is specially designed for cutting samples embedded in Technovit 7100 or 8100. The blade holder can be clamped in the reusable blade holder of commercially available microtomes.

Embedding in Technovit 7100

Manufacturer: Kulzer GmbH, **Resin type:** HEMA (Hydroxy-ethyl-methacrylat)
Suitability: non mineralized soft and firm tissues, **Fixatives:** formalin, PFA, glutardialdehyde, alcohol, Schaffer
Post-treatment: Remove fixative according to usual regulations, if necessary water and raise to 70 % ethanol

Infiltration steps		Duration	Process
Times apply to sample sizes of approx. 5 mm^3 => double times for each additional 5 mm^3			
Ethanol 70 %		2 - 3 h	Dehydration
Ethanol 80 %		2 - 3 h	Dehydration
Ethanol 90 %		2 - 3 h	Dehydration
Ethanol 96 %		2 - 4 h	Dehydration
Ethanol 96 %		2 - 4 h	Dehydration
Pre-infiltration solution I • 100 ml Basic solution Technovit 7100 • 100 ml Ethanol 96 %	mix at a ratio of 1 : 1	3 - 6 h	Infiltration
Infiltration solution • 100 ml Basic solution Technovit 7100 • 1 g Hardener 1 (= 1 Bag)	add slowly while stirring, let stir for at least 30 minutes	8 - 24 h	Infiltration (for larger samples possibly several identical stages in succession)
Pouring and curing			
Pouring solution / embedding solution **Stock solution:** • 100 ml Basic solution Technovit 7100 • 1 g Hardener 1 (= 1 Bag)	add slowly while stirring, let stir for at least 30 minutes	**a few hours, depending on size:** **< 5 ml: 1 h ~20 °C then 1h ~37 °C** **> 5 ml: bei ~4 °C**	Embedding of the samples into Teflon or plastic moulds Polymerize according to the specifications on the left
Working solution: • 15 ml Stock solution (o. Multiple) • 1 ml Hardener 2 (o. Multiple)	stir for a few minutes, **use immediately**		
prepare a maximum of 45 ml, otherwise it`ll cure too quickly			
sawing / grinding, cutting, milling			
Diamond band saw / inner diameter: • Cutting-grinding techniques are not possible: resin too soft	**Rotary microtome:** • D-blade (carbide) or Kulzer Histoblade • Cutting thickness: 1 - 10 µm • Sectioning and stretching with Aqua dest.	**Ultra-milling machine:** • Limited use possible with milling head for soft samples	
Duration of infiltration: approx. 2 - 8 days (depending on sample size)			

Staining of Technovit 7100 sections

Technovit 7100 sections can be stained with many histological staining methods to differentiate tissue.

When histological staining Technovit 7100 sections, it must be taken into account that the resin itself cannot be removed. Therefore, only the actual cut surface is available as a reaction component for the staining, and not the entire tissue section, as is the case with deparaffined or deplastisized sections. This has consequences for the staining:
On the one hand, longer staining times are necessary, on the other hand, some stains stain the plastic itself. In addition, some stains or pickling solutions or the liquid base of the dyes (alcohols) can attack the resin itself and possibly make the sections unusable. Therefore, the desired stainings should always be established on test samples and incubation times in the individual solutions should be kept as short as possible.

Application / Procedure

The slides with the sections are immersed directly into the first staining solution ***when dry, without further pre-treatment.*** Pre-treatment in alcohol or water is not necessary!

Implementation

The staining is done according to the usual procedure for histological staining. First, fine structures (e.g. cell nuclei) are stained, then cell plasma, and finally fibres and basic substance. The staining times depend on the desired result and must be determined empirically for each type of sample.

Exemplary staining protocol H&E:

Use dry sections!

Step 1: Hematoxylin after Gill III	5 min
Step 2: Blueing in tap water	10 min
Step 3: Rinse in aqua dest	short
Step 4: Eosin alcoholic	2 - 5 min
Step 5: Ethanol 96 %	1 min
Step 6: Ethanol 99 %	1 min
Step 7: Ethanol 99 %	1 min
Step 8: Xylene	1 min
Step 9: Xylene	1 min
Step 10: Coverslipping	

Suitable and unsuitable staining

From the broad spectrum of histological staining, almost all aqueous and alcohol-based staining solutions can be used. Certain difficulties arise with strongly acidic staining solutions or pickling solutions with phosphotungstic or phosphomolybdic acid, if long pickling times are necessary.

Post-treatment and coverslipping

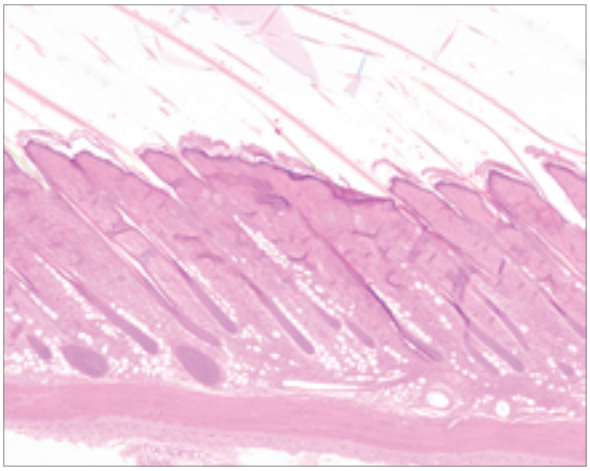
Finished stained sections are dehydrated in the usual way over an alcohol series and then coversliped after 1-2 xylene steps. However, the different compositions of the mounting media often lead to subsequent reactions. It is recommended to test the compatibility of the mounting media before using them.

Tested and coordinated staining kits:

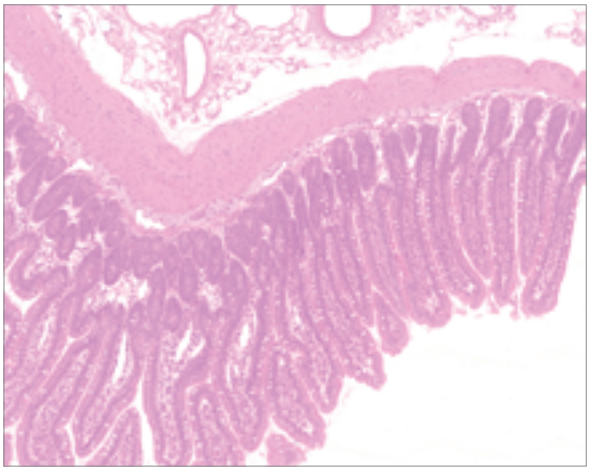
Many of our staining kits and individual solutions are suitable for staining Technovit 7100.

Catalog no.	Description
12156	Hematoxylin & Eosin (H&E)
12153	PAS reaction
15631	Feulgen staining
11097	Prussian blue

Stainings examples of Technovit 7100 sections



Rat, Skin, Hematoxylin & eosin, Catalog no.: 12156



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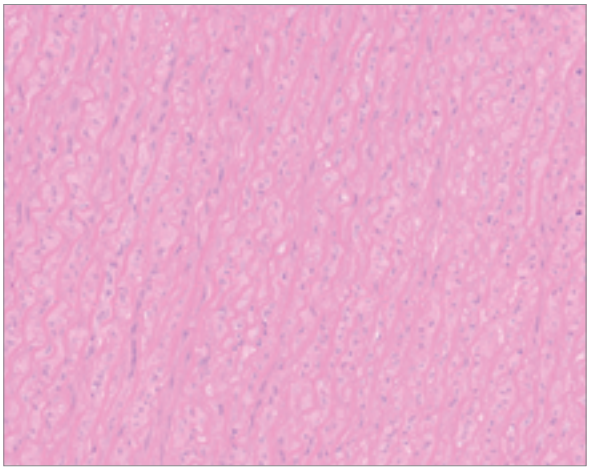
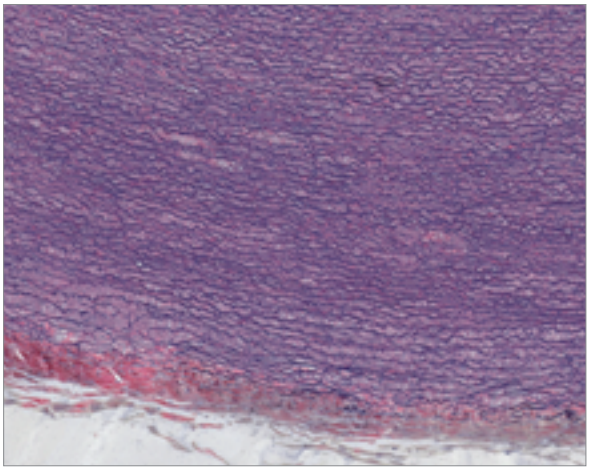
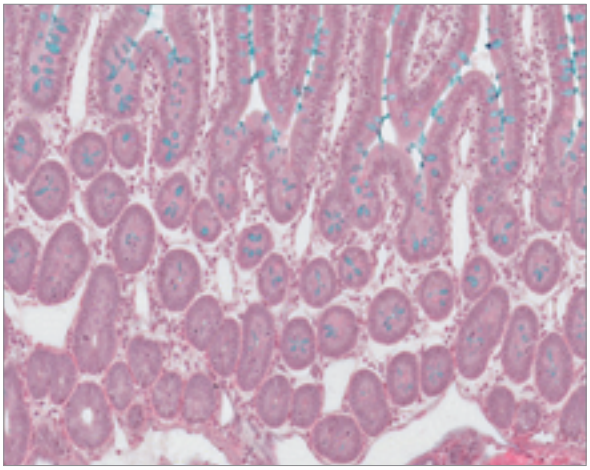


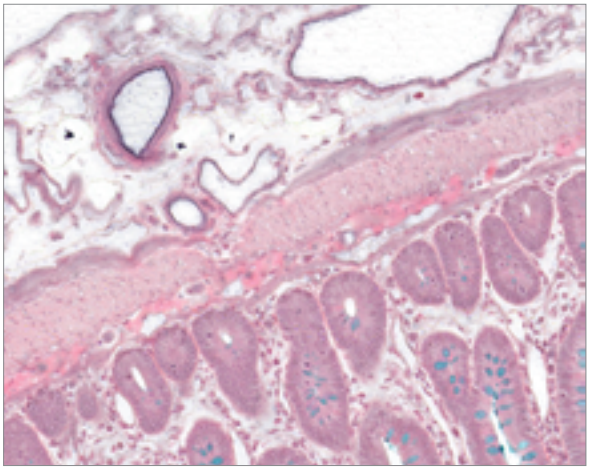
Rat, Aorta, Hematoxylin & Eosin, Catalog no.: 12156



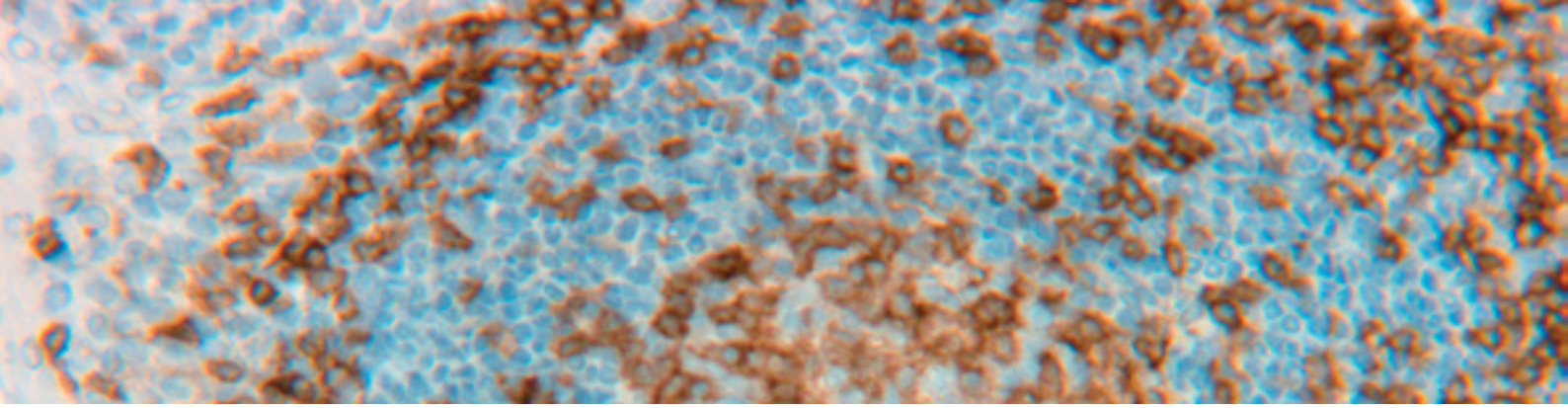
Rat, Aorta, Movat Pentachrom after Verhöff, Catalog no.: 12061



Rat, Intestine, Movat Pentachrom after Verhöff, Catalog no.: 12061



Rat, Aorta, Movat Pentachrom after Verhöff, Catalog no.: 12061



Technovit® 8100

Sections specifically for immunohistochemistry

Technovit 8100 is a plastic embedding system based on HEMA for light microscopic examinations. It is suitable for embedding all tissues in medicine, zoology and botany. Sections of non-decalcified or short decalcified iliac crest biopsies and implanted biomaterials can be used not only for histological staining but also for enzyme and immunohistochemistry.

Material properties

Technovit 8100 is a combination of an almost odourless plasticizer and a hydrophilic resin. Technovit 8100 has been specially developed for cold polymerisation (4 °C).

During the curing process, the embedding mould must be absolutely airtight, as the polymerisation system is sensitive to oxygen.

The advantages at a glance

- Reproducibility and reliability of the embeddings through constant and documented quality controls of the individual components.
- Low polymerisation temperature of 0 °C - 10 °C by the special catalyst system and the Teflon moulds.
- Homogenous hardness of the block, resulting in even, very thin cuts.
- Low shrinkage artifacts, therefore excellent tissue morphology.
- Routine staining, enzyme detection and immunohistochemistry possible.
- Hematological iliac crest biopsies do not need to be decalcified.
- Low toxicity through special combination of plasticizer and catalyst system.

Application

Apply Technovit 8100 according to the step by step instructions. Place the fixed and dehydrated samples in the infiltration solution. A low temperature and agitation of the samples during the entire embedding process are advantageous.

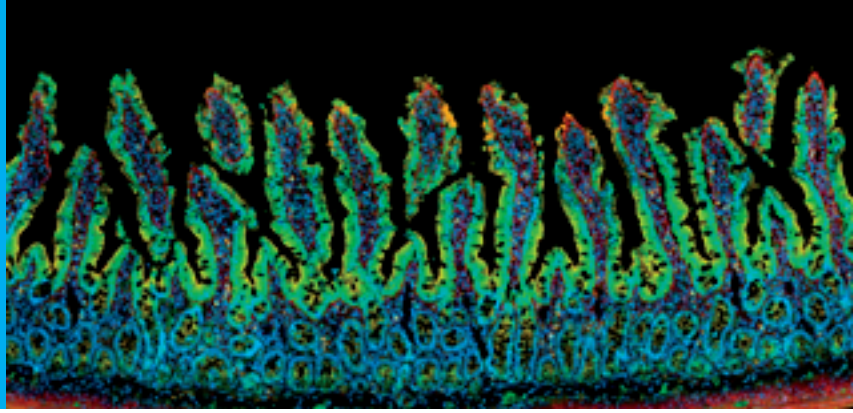
Polymerisation

Prepare the polymerisation mixture according to the instructions and fill it into the embedding moulds. Align the infiltrated samples in them. Cover the wells airtight with foils. For curing, place the embedding form on a pre-cooled gel plate or thin layer of ice at 4 °C.

After the polymerisation is complete, remove the foils and block them with Histobloc and Technovit 3040.

It is not possible to remove the resin before staining or reaction.

Sections with Technovit 8100 can be used not only for histological staining but also for enzyme and immunohisto-chemistry.



Product data

Catalog no.	Description	Content
12226.K0500	Technovit 8100 Combipack	500 ml Basic solution 5 x 0,6 g Hardener 1 1 x 30 ml Hardener 2 1 x 500 pcs. PE-Foils

Technical data	
Colour	transparent
density = specific weight g/cm3 (DIN 53479)	1,08
Refractive index monomer polymer	 1,4485 1,4990
Storage temperature	max. 25 °C
Shelf life	2 years

The processing

The following instructions for fixation and dehydration are not mandatory. Technovit 8100 can also be infiltrated and polymerised after another pretreatment.

For the entire process use tightly closing glass or PE disposable vessels (approx. 20 ml)!

TIP:
During fixation, dewatering and infiltration the samples must be moved constantly!

Fixation

To achieve optimal immunohistochemical results, it is recommended to work at 4 °C during the entire embedding procedure and to aim for short fixation times. Fix the smallest possible tissue pieces (1 mm thickness) in 2 % paraformaldehyde in phosphate buffer pH 7.4 at 4 °C for 3 to 4 hours. Afterwards Post-treat for 12 hours (overnight) in phosphate buffer pH 7,4 with an addition of 6,8 % sucrose at 4 °C.

Dehydration

Dehydrate the tissue in cold acetone (100 %) for at least for 1 hour at 4 °C. During the first few minutes, change frequently until the acetone remains clear.

Infiltration

- Technovit 8100 Basic solution 100 ml
- + Technovit 8100 Hardener 1 1 Bag, 0,6 g

Dissolve in a clean PE or glass vessel free of detergent and then store at 4 °C. The infiltration solution is stable for a maximum of 4 weeks at 4 °C when sealed.

Transfer sample from the acetone directly into the pre-cooled infiltration solution. The samples remain in this solution at 4 °C for 6 - 10 hours.

Polymerisation

- Infiltration solution, 4 °C 15 ml
- + Technovit 8100 Hardener 2, 4 °C 0,5 ml

Measure with commercially available pipetting aids and mix well in a PE or glass vessel. Add the infiltrated samples to this polymerisation mixture, close the vessel and continue mixing carefully for about 5 minutes.

The color of the polymerisation solution initially changes yellowish-greenish, but becomes colorless after curing.

Using a disposable pipette, completely fill the wells of the Histoform, orient the tissue in it and immediately cover it with the supplied transparent PE foil. In order to seal the mould airtight, several films can be used for one mould. Do not squeeze out bubbles, but add polymerisation solution and lay on new film. During the polymerisation (at least 3 hours), the embedding mould must be placed on a cooling plate or thin layer of ice at 4 °C. Do not bring the mould and samples into contact with moisture.

Maximum polymerisation temperature depending on the ambient temperature

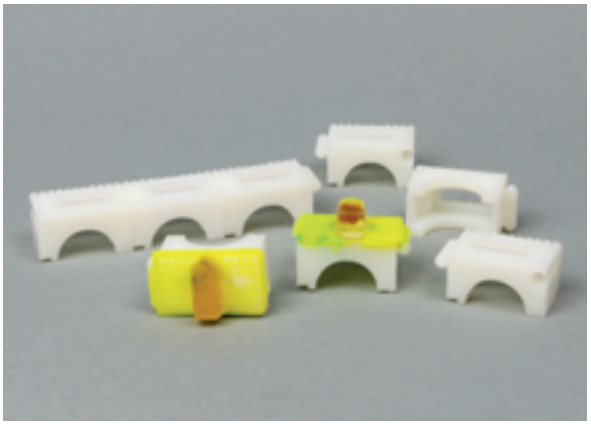
Histoform Q

Material	Room temp. ca. 20 °C	Refrigerator 4 °C	Refrigerator on ice 0 °C
Technovit 8100 30:1	—	69 °C	48 °C
Technovit 8100 35:1	—	52 °C	42 °C

Maximum polymerisation temperature depending on the ambient temperature

Histoform S

Material	Room temp. ca. 20 °C	Refrigerator 4 °C	Refrigerator on ice 0 °C
Technovit 8100 30:1	69 °C	21 °C	12 °C
Technovit 8100 35:1	67 °C	19 °C	11 °C



Preparation of solutions at a glance

Solution	Ethanol	Basic solution Technovit 8100	Hardener 1 Technovit 8100	Infiltration-solution	Hardener 2 Technovit 8100	Application-temperature
Infiltration		100 ml	0,6 g (1 Bag)			4 °C
Polymerisation S				15 ml	0,5 ml	4 °C on ice

Blocking out, further processing, archiving

Technovit 3040

To get the polymerised blocks out of the Teflon moulds, the Histobloc carrier parts are glued to the polymerised blocks with another Technovit resin and then pulled out of the mould.

Technovit 3040 is a fast-curing 2-component resin based on methyl methacrylate (MMA) which, due to its chemical composition, forms a stable, permanent bond with the cured block Technovit 7100 (as well as Technovit 8100 or Technovit 9100).

Histobloc - carrier parts

The Histobloc carriers are adaptable for the Histoform S and Q or also for the Histoform N and can be clamped firmly in all microtomes with universal holder.

Procedure

After complete polymerisation, the Histobloc carrier part is placed in the recess provided in the Histoform S/Q Teflon embedding mould.

Mix Technovit 3040 as viscous as possible (mixing ratio around 3 : 1 / powder : liquid) and pour the mixture into the recess of the Histobloc.

Depending on room temperature, Technovit 3040 is cured in 5 - 10 minutes and the histobloc is firmly bonded to the sample.

The sample can now be removed from the mould and is ready to be cut. Blocking up with the very hard Technovit 3040 stabilises the elastic Technovit 7100 or Technovit 8100 in such a way that even the thinnest cuts are possible.

Further processing

Clamp the blocks firmly in the jaw clamping device on the rotary microtome. To cut the blocks, use a Histoblade (in combination with the Kulzer knife holder) or carbide knife (note the knife angle). Remove the cut dry with tweezers and apply to a stretching bath (aqua dest.). Mount **on coated slides** and dry for 2 hours or longer at 37 °C. Dry sections not immediately required (for immunohistochemistry) at room temperature and store at 4 °C for a maximum of 5 days.

Before staining or immunoassaying, the sections must be dried at 37 °C for at least 2 hours. Then place the sections directly into the staining solution or start with the enzymatic pretreatment (see page 18).

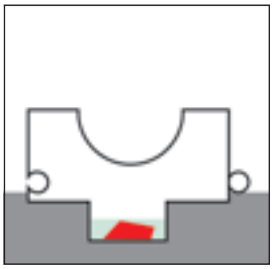
Slide coating

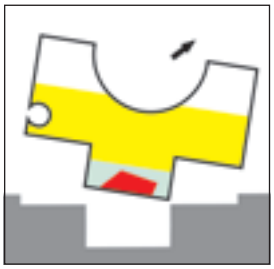
For example, immerse slides for 15 minutes in a solution of 0,5 % alcian blue at 65 °C or coat the slide with 0,1 % poly-L-lysine liquid. All commercially available coated slides can be used.

Storage and archiving of samples

The Histoblocs can be plugged together for better archiving. This enables consecutive numbering and space-saving storage.

For histochemical and immunohistological investigations it is recommended to store the blocks in PE bags at +4 °C or longer even at -20 °C.





Cutting on the microtome

Technovit 8100 samples can be cut on commercially available microtomes in thicknesses down to 1 µm. D-ground blades in a carbide design are used for this purpose. Normal D-knives can be used, but do not provide optimal results.
Disposable carbide knives are only suitable if they are designed as a version especially for cutting resins.

The **disposable Histoblade** from Kulzer is such a carbide blade which is especially suitable for cutting tissue samples embedded in HEMA (2-hydroxyethyl methacrylate) as well as materials in industrial applications up to 1 µm cutting thickness. Together with the stable blade holder, this provides a low-cost alternative to other blades that can be used in all commercially available microtomes.

For this purpose there are different blade holders available:

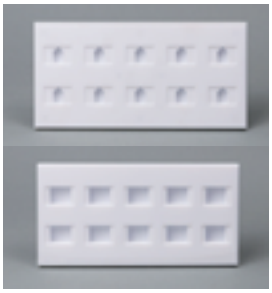
- Knife holder „NR“ or „SL“ for Microm/Thermo-Microtomes; Knife angle adjustment 9°
- Knife holder „N“ for Reichert-Junge/Leica-Microtomes; Knife angle adjustment 0°

The advantages::

- Heavy, stable design
- Easy handling
- Good sectioning results up to 1 µ cutting thickness
- Optimized cutting quality
- Improved service life

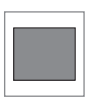
Dimensions:
Histoblade: 60 x 19 x 1 mm, 2 holes à 4 mm
Knife holder: 170 x 34 x 10 mm

Accessories and supplements



Histoforms S & Q:
Special embedding moulds made of Teflon with stainless steel bottom for heat dissipation with 10 wells.

Histoform S
Dimensions: W x H x D: ca. 10 x 16 x 6,5 mm.



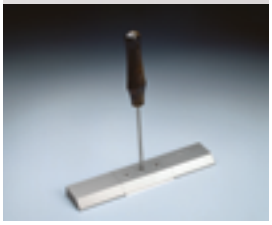
Histoform Q
Dimensions: W x H x D: ca. 20 x 16 x 10 mm.

Histobloc - Carriers
The Histobloc carriers are adaptable for the histoform S + Q, as well as for the histoform N or N+ (Technovit 9100).

Mixing spatula
Wood spatula for mixing Technovit 3040.

Technovit 3040
Fast curing 2-component resin for blocking and fixing of cured samples on Histoblocs.

Mixing cup
Paper cup for mixing Technovit 3040.



Histoblade und Knife holder
Histoblade is specially designed for cutting samples embedded in Technovit 7100 or 8100. The blade holder can be clamped in the reusable blade holder of commercially available microtomes.

Embedding in Technovit 8100

Manufacturer: Kulzer GmbH, **Resin type:** HEMA (Hydroxy-ethyl-methacrylat)
Suitability: soft tissue, slightly hard tissue, immune staining, **Fixatives:** formalin, PFA

Infiltration steps		Duration	Process
Times apply to sample sizes of approx. 1-2 mm => double times for each additional mm³			
Acetone, cold (4 °C)		1 - 3 h	change several times until the acetone remains clear
Acetone, cold (4 °C)		1 - 3 h	
Acetone, cold (4 °C)		1 - 3 h	
Infitration solution • 100 ml Basic solution Technovit 8100 • 0,6 g Hardener 1 (= 1 Bag)	add slowly while stirring, let stir for at least 30 minutes	4 - 24 h	Infiltration
Pouring and curing			
Pouring solution / embedding solution **Stock solution:** • 100 ml Basic solution Technovit 8100 • 0,6 g Hardener 1 (1 Beutel)	add slowly while stirring, let stir for at least 30 minutes	a few hours, depending on size: **< 5 ml: 1 h room temperature, then 1 h 40 °C** **> 5 ml: refrigerator 4 °C**	embed the samples into Teflon or plastic moulds, cover the surface with PE film to make the mould airtight. Allow to polymerise for at least 3 h at 4 °C on a cooling plate or in the refrigerator.
Working solution • 15 ml Stock solution (o. Multiple) • 0,5 ml Hardener 2 (o. Multiple)	leave to stir for a few minutes		
		The polymerisation solution first becomes yellowish-greenish, but then colourless after curing	
Block out			
Sticking on HistoBlocks **Technovit 3040:** • Technovit 3040 Powder • Technovit 3040 Universal Liquid	Mix and blend in the ratio 1 : 1		
Process immediately, always produce only small quantities			
sectioning, sawing, grinding			
Rotation saw: • not possible, too soft	**Hard-sectioning microtome:** • Cutting thickness: 1 - 10 µm • sectioning with water	**Stick on slide:** • Stretching with water on e.g. Superfrost Plus slides	
Duration of infiltration: approx. 2 - 8 days (depending on sample size)			

Staining of Technovit 8100 sections

In contrast to Technovit 7100, Technovit 8100 slices can not only be stained with many histological staining methods, but can also be treated with many immunohistochemical detection methods.
As with Technovit 7100 sections, it should be noted that Technovit 8100 cannot be removed from the specimens. Thus, only the actual section surface is available as a reaction component for the staining or immune reactions, and not the entire tissue section, as is the case with deparaffined or deplastisized sections. This has consequences for the immune reactions: On the one hand, longer incubation times are necessary, on the other hand, some antibodies adhere to the resin itself. In addition, some reagents can attack the resin itself and possibly render the sections unusable. Desired immune detection should therefore always be established on test samples and the incubation times in the individual solutions should be kept as short as possible.

Preparation

The slides with the sections are exposed to the enzymatic antigen retrieval **dry, without further pre-treatment** directly. Pre-treatment in alcohol or water is not necessary!

Procedure

Immunohistological staining is performed according to the usual procedure for an immune reaction. Counterstaining according to classical histological protocols is possible in the same way as with Technovit 7100: First, fine structures (e.g. cell nuclei) are stained, followed by cell plasma, and finally fibres and basic substance. The staining times depend on the desired result and must be determined empirically in each individual case (sample type).

An exemplary IHC protocol

- Enzymatic pretreatment: incubate for 5 to 10 minutes in 0,01 % trypsin with 0,1 % CaCl2 (calcium chloride) pH 7,8 (37 °C).
- Wash out several times in phosphate buffer (PBS) for 5 minutes.
- Incubate for 2 hours at 37 °C with primary antibody.
- Blocking of the endogenous peroxidase with 0.06% hydrogen peroxide in phosphate buffer (PBS) (30 minutes at room temperature).
- Wash out several times in phosphate buffer (PBS) for 5 minutes.
- Incubate 30 minutes at room temperature with the secondary antibody.
- Wash out several times in phosphate buffer (PBS) for 5 minutes.
- incubate with Diaminobenzidine (DAB)
- 10 - 15 seconds counterstain with hematoxylin..
- Blue under tap water for 3 minutes.
- Coverslip with e.g. glycerine gelatine.

Immune detection is possible with the AP, PAAP, APAAP, ABC (avidin-biotin or strept avidin-biotin) and polymer conjugated methods.

It is not recommended to use a detergent e.g. Tween in the rinsing buffer. The peroxidase should be dissolved in buffer.

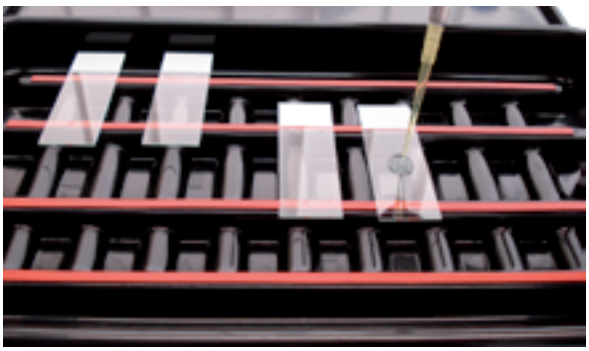
Notes

With the constantly changing range of new products for histochemistry and immunohistochemistry, it is always advisable to follow the respective manufacturer's instructions.

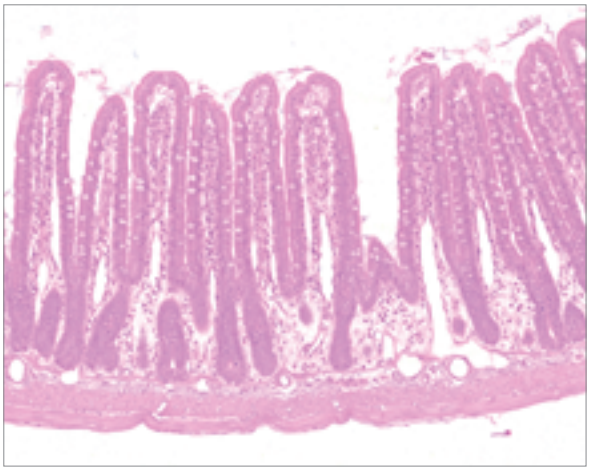
Post-treatment and coverslipping

Dehydrate and cover ready stained sections over alcohol series. It is recommended to test the compatibility of a mounting medium before using it.

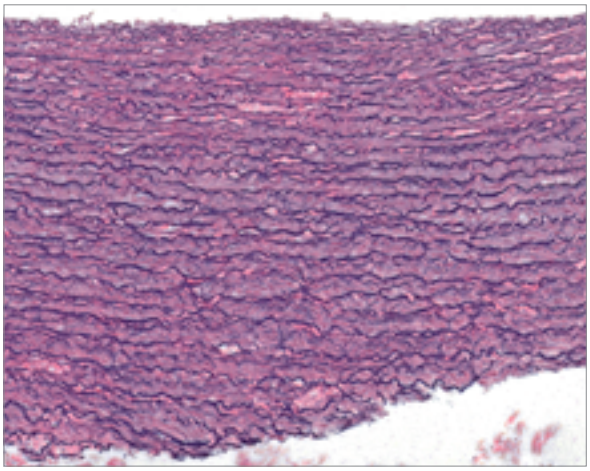
Catalog no.	Description
12156	Hematoxylin & Eosin (H&E)



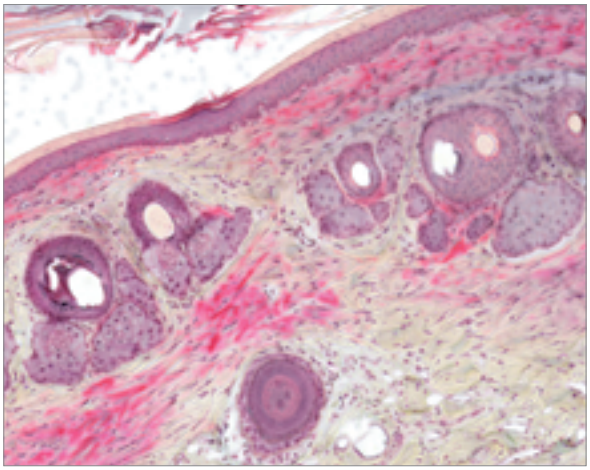
Stainings examples of Technovit 8100 sections



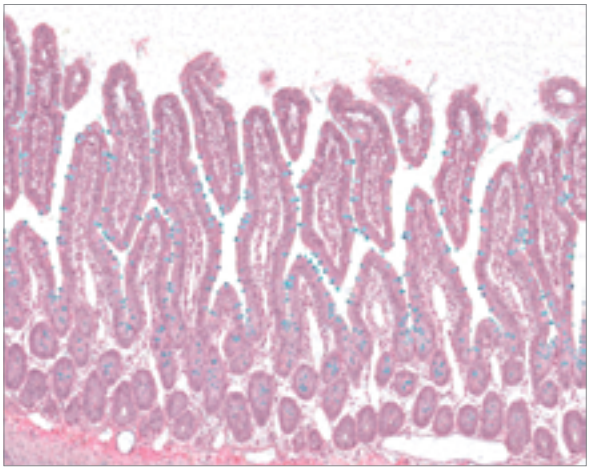
Rat, Intestine, Hematoxylin & Eosin, Catalog no.: 12156



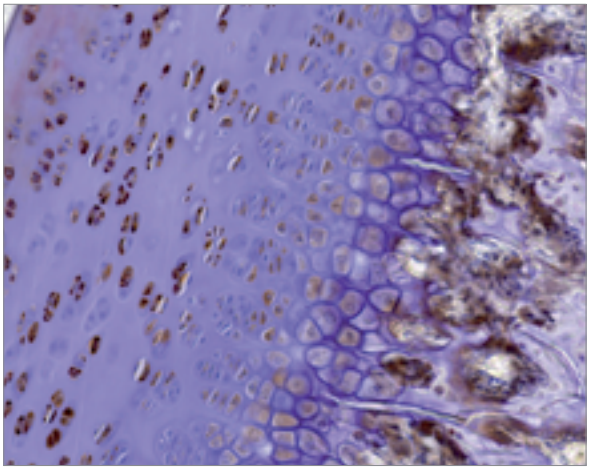
Rat, Aorta, Movat Pentachrom after Verhöff, Catalog no.: 12061



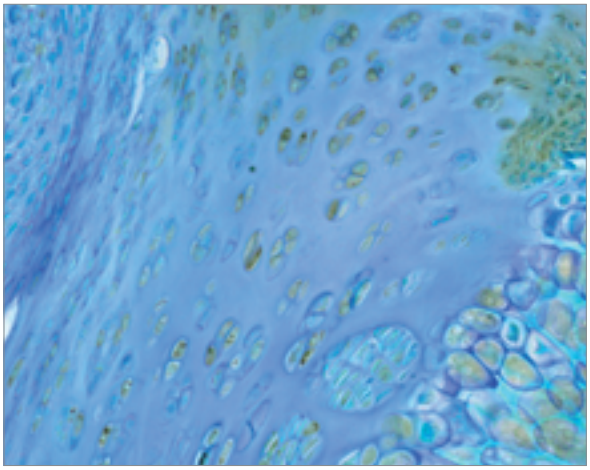
Rat, Tail, Movat Pentachrom after Verhöff, Catalog no.: 12061



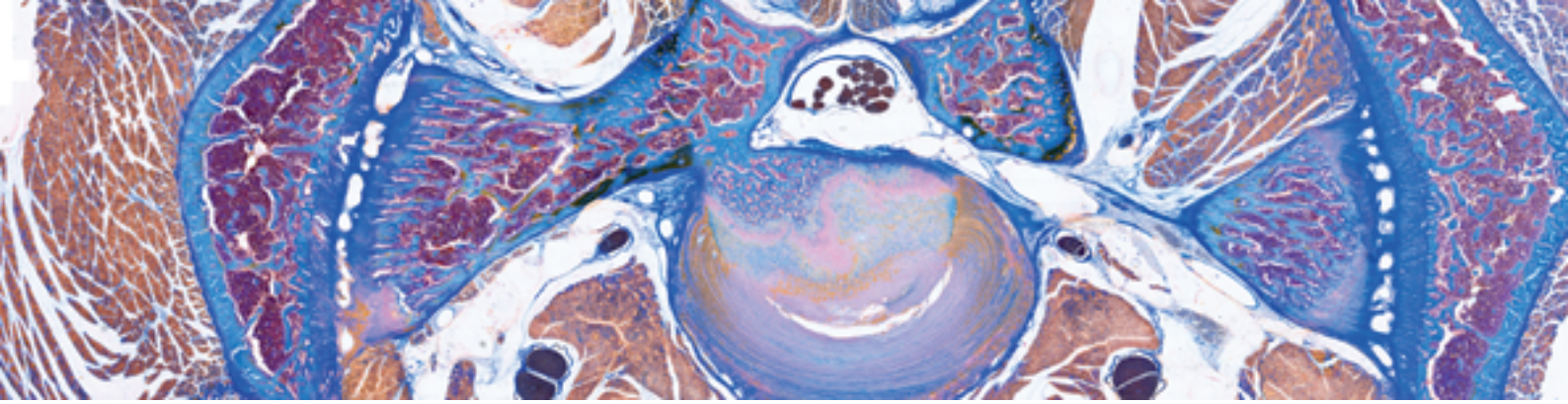
Rat, Intestine, Movat Pentachrom after Verhöff, Catalog no.: 12061



Rat, Joint; IHC vs. osteocalcin, Hematoxylin counterstain



Rat, Joint; IHC vs. osteocalcin, Methylene blue counterstain



Technovit® 9100

Sections and cut & ground thin sections

Technovit 9100 is a resin embedding system based on MMA (methyl methacrylate).
Technovit 9100 is used in medicine, botany and zoology.

Technovit 9100 was specially developed for embedding mineralised tissue, but also soft tissue with an extended examination spectrum in light microscopy. The deplatinated sections are suitable for histological overview staining, enzyme and immunohistological analysis, including in-situ hybridization.

Thin sections for immunohistology can be glued to glass slides and deplastinated.

Applications

- **Hard-cutting technique for the production of thin sections**
  Examples: Iliac crest biopsies, smaller spongios and compact bone tissue samples
- **Cutting-grinding technology (cutting process in point contact technology)**
  Examples: Tooth and jaw segments with and without implant, non-cemented endoprostheses with shaft bone
- **Combined cutting-grinding technique and hard cutting technique (target preparation)**
  Examples: Interface and environmental assessment for metal implants and non-cemented endoprostheses

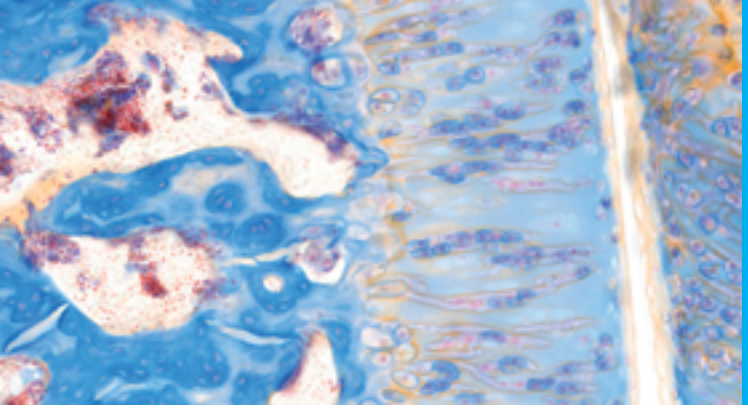
Tissues that cannot be cut are tooth-bearing jaw sections with fillings, crowns and bridges, thick corticalis, implant-bearing (metal or ceramic) jaw or tubular bones or even brittle, hypermineralised bone parts.

Material properties

The polymerisation of hydrophobic Technovit 9100 takes place under exclusion of oxygen, with the aid of a catalyst system consisting of peroxide and amine. Additional components such as PMMA powder and regulators enable controlled polymerisation at low temperatures (in the range of -2 °C to -20 °C, depending on the volume), which guarantees complete heat dissipation.

The advantages at a glance

- Polymerisation at temperatures below zero.
- Reproducibility of the embedding results.
- Reliability through constant and documented quality controls.
- Uniform hardness of the block..
- Durable transparency of the PMMA block.
- Better cutting and staining results due to contained hydrophilizing agent.
- Can be used for thin-cutting as well as sawing and grinding techniques.
- Enzyme and immunohistology and in situ hybridization possible (thin sections & cut and ground sections).



Technovit 9100
has been specially developed for mineralized tissue and is used for specimen for hard cut or cutting-grinding technique.

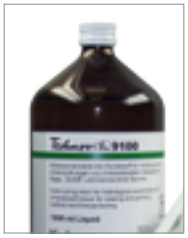
Product data

Catalog no.	Description	Content
12225.K1000	Technovit 9100 Combipack	1000 ml Basic solution 120 g PMMA-powder 8 x 1 g Hardener 1 1 x 10 ml Hardener 2 1 x 5 ml Regulator
12225.05000	Technovit 9100 Base liquid	5000 ml
12225.F1000	Technovit 9100 PMMA-powder	1000 g
12225.F0010	Technovit 9100 Hardener 1	10 x 1 g
12225.00010	Technovit 9100 Hardener 2	1 x 10 ml
12225.00005	Technovit 9100 Regulator	1 x 5 ml

Description	Quantity	Components
Technovit 9100 Basic solution stabilized	1 x 1000 ml	1
Technovit 9100 PMMA-powder	120 g	2
Technovit 9100 Hardener 1	8 Bags á 1 g	3
Technovit 9100 Hardener 2	10 ml	4
Technovit 9100 Regulator	5 ml	5

The components

Technovit 9100 Basic solution - Component 1



The basic solution Technovit 9100 consists of stabilized MMA. The hydrophilicity is improved by adding a suitable hydrophilizing agent. Technovit 9100 Base Solution can be used both stabilised and de-stabilised.

Technovit 9100 PMMA powder - Component 2

The PMMA powder is used to ensure lower polymerisation shrinkage, a reduction of the released heat of polymerisation and a better polymerisation process.

Technovit 9100 Hardener 1 - Component 3

Hardener powder 1 is a peroxide compound which induces polymerisation in combination with hardener 2.

Technovit 9100 Hardener 2 - Component 4

Hardener liquid 2 has a catalytic effect on hardener 1 to enable targeted polymerisation even at very low temperatures below 0 °C.

Technovit 9100 regulator - component 5

This consists of a reactive organic compound, which enables controlled polymerisation with controlled low temperature peaks even with large polymerisation quantities.

The processing

Fixation - pre-treatment of the tissue

Fixation is carried out in the respective fixative for at least 12 - 48 hours, depending on the size of the tissue and the antigen/enzyme to be detected.

Suitable fixatives for subsequent embedding in Technovit 9100 are:

a) 4 - 10 % neutral or buffered formalin solutions
b) Fixative according to Schaffer (formol/alcohol)
c) Paraformaldehyde solution of various concentrations or pH values
d) Glutardialdehyde fixing solutions

Fixatives containing picric acid, such as Bouin's solution, are not suitable because picric acid residues affect the polymerisation of MMA.

Dehydration and infiltration

The processing may only be carried out in glass or polyethylene (PE) containers!

Dehydration is carried out in an ascending alcohol series (dehydration machine) at room temperature. In an insufficiently dehydrated tissue, so-called blowholes are formed, which consist of white bead polymer and negatively affect the cutting as well as the cutting quality. Xylene is used as an intermedium.

Infiltration with Technovit 9100 takes place in 4 steps via three so-called pre-infiltrations and one infiltration step. Some automatic machines allow the use of step 1 and 2 (ATTENTION: Observe manufacturer's instructions!). The times indicated are minimum times and refer to small spongious and cortical bone tissue samples and iliac crest biopsies. For larger tissue samples, the times and volume must be adjusted and suitable times need to be determined empirically if necessary.

Pre-infiltration and infiltration solutions can be stored for several weeks after preparation, if they are kept in the refrigerator or freezer. If the solutions have been prepared with de-stabilized base solution, the shelf life will be shorter. Discoloured solutions, especially those with a slightly pink discolouration, must be discarded and disposed according to the instructions in the MSDS.

Dehydration, Intermedium and Pre-infiltration

Phase	Solution	Concentration	Time / Temp.
Dehydration 1	Ethanol	70%	> 1 h / RT
Dehydration 2	Ethanol	80%	> 1 h / RT
Dehydration 3	Ethanol	96%	> 1 h / RT
Dehydration 4	Ethanol	96%	> 1 h / RT
Dehydration 5	Ethanol	abs.	> 1 h / RT
Dehydration 6	Ethanol	abs.	> 1 h / RT
Dehydration 7	Ethanol	abs.	> 1 h / RT
Intermedium 1	Xylol		> 1 h / RT
Intermedium 2	Xylol		> 1 h / RT
Pre-infiltration 1	Xylol/ Technovit 9100 Basis, (stab.)	1:1	> 1 h / RT
Pre-infiltration 2 (last phase in machine)	Technovit 9100 Basis, (stab.) + Hardener 1		> 1 h / RT
Pre-infiltration 3 (Refigerator)	Technovit 9100 (destab.) + Hardener 1		> 1 h / 4 °C
Infiltration (Refigerator)	Technovit 9100 (entstab.) + Hardener 1 + PMMA-powder		> 1 h / 4 °C after 5 days change solution

De-stabilization of the base solution - Processing the components

The basic solution Technovit 9100 can be used both stabilised and de-stabilised. The use of de-stabilized base solution guarantees the result for all immunohistochemical analysis analogous to paraffin histology.

Fill the chromatography column with approx. 50 g Al_2O_3 (active, basic, 90) and slowly run Technovit 9100 Base Solution (material number 1) through the column. One column filling with Al_2O_3 is sufficient to destabilise 3 - 4 litres of the base solution. The destabilized solution is portioned into sealable brown glass bottles and stored at 4 °C for continuous processing (max. 5 days) or aliquoted at -15 °C to -20 °C. From pre-infiltration step 3 onwards, it is possible to work with de-stabilized base solution. When working with de-stabilized MMA base solution, the smaller amount of peroxide can be used for infiltration solution and stock solution A.

Preparation of the working solutions							
Component no.	1	2	3	4	5		
Name	Basic solution	PMMA-powder	Hardener 1	Harde-ner 2	Polymeri-sations-regulator	Processing temperature	Storage shelf life
Pre-infiltration 1	Mix 200 ml base with 200 ml xylene					Room-temperature	1/2 year at –20 °C
Pre-infiltration 2	200 ml		1 g			Room temp.	1/2 year at –20 °C
Pre-infiltration 3	200 ml		1 g			Room temp.	1/2 year at –20 °C
Infiltration solution	ad 250 ml	20 g	1 g / 2 g*			4 °C	1/2 year at –20 °C
Stock solution A	ad 500 ml	80 g	3 g / 4 g*			4 °C	1/2 year at –20 °C
Stock solution B	ad 50 ml			4 ml	2 ml	4 °C	1/2 year at –20 °C

Preparation of the solutions

- **Stock solution**
  Preparation of pre-infiltration, infiltration and stock solutions according to the exact instructions in the manual for Technovit 9100 or the table above. Observe storage temperatures!
- **Polymerisation solution**
  The cooled stock solutions A and B should only be mixed immediately before use in the ratio of 9 parts stock solution A and 1 part stock solution B (v/v) in a beaker using a glass rod.

Polymerisation

The polymerisation mixture is poured into the pre-cooled embedding mould. Orientate the infiltrated tissue in it, pour the polymerisation mixture over the entire edge and then evacuate. The evacuation can be carried out either in the pre-cooled desiccator at 4 °C (slight vacuum, e.g. water jet pump or vacuum pump at 200 mbar) or in the freezer with externally connected vacuum pump for approx. 10 minutes. ***Then seal the mould airtight so that no contact with oxygen can take place!***

The polymerisation depends on the volume of the embedding moulds and takes place in the range of -2 °C to -15 °C; the polymerisation should be completed after approx. 24 hours.

The larger the volume of the embedding mould, the lower the ambient temperature must be! The cooling capacity of the explosion-proof cooling device used (freezer compartment in the refrigerator, freezer, deep freezer, freezer tray e.g. for paraffin blocks with lid closure) must be taken into account. Reproducible results for different sample sizes are obtained in a freezer with variably adjustable temperatures between -2 °C and -25 °C at a temperature stability of +/- 0.5 °C. ***Do not open the moulds during polymerisation!***

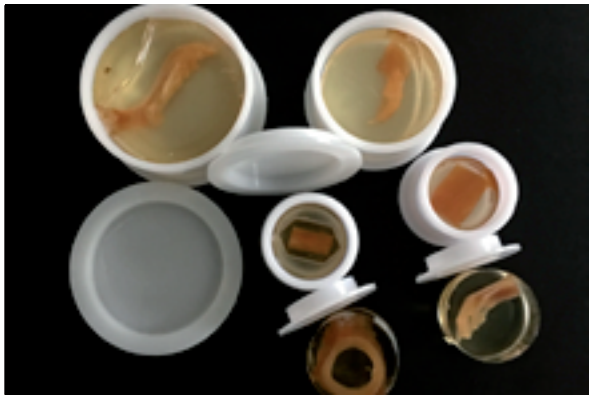
Polymerisation temperatures and times		
Volume of the embedding mould	Temperature window	Duration
bis 5 ml	-2 bis -4 °C	24 h
5 - 15 ml	-4 bis -8 °C	24 - 48 h
15 - 25 ml	-8 bis -15 °C	24 - 48 h
über 25 ml	-12 bis -20 °C	24 - 48 h

For particularly large samples (endoprostheses), a commercial PMMA granulate can be used as filling material. This reduces the amount of liquid polymerisation solution required.

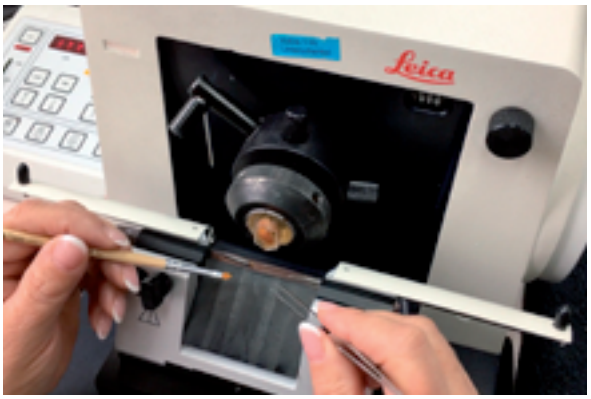
Blocking

If the samples have warmed to room temperature after curing, use the Histobloc N and Technovit 3040 to block the Histoform N. First loosen the screw and remove the lid and foil. The block is tightly clamped in the standard clamps on the rotation microtome for hard cuts.

When using the round histo-moulds, the lid and bottom are removed and the specimen is pushed through. It can then be used directly in the round specimen holder on the rotation microtome for hard sections.



Polymerized sample in embedding form



Polymerized sample in round sample holder

Processing of the polymers

Depending on the question, further processing is carried out by hard-cutting technique on suitable microtomes with sample retraction or by cutting-grinding technique on suitable diamond saw systems (diamond band saws, internal hole saws, circular saws) and grinding devices (plate grinding devices with material removal measurement). Processing with ultra-milling or wet grinding machines is also possible.

Cutting-grinding technique:

- Cutting in point contact technology (CP technology from EXAKT) as well as grinding in surface or line contact process with corresponding devices (e.g. EXAKT company).

Hard-cut technique:

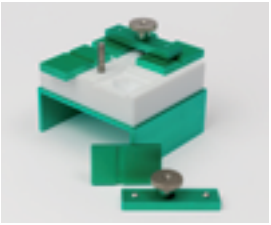
- Production of hard sections with corresponding hard cut microtomes (microtome with sample retraction) and use of hard metal knives with D-grinding or tungsten carbide blades.
- Production of semi-thin sections using glass and diamond knives. The blocks are trimmed beforehand.
- Use 30 % ethanol („cutting fluid") to cut the Technovit 9100 blocks.
- Place sections on coated slides, stretch with 50 % ethanol („stretching liquid") and cover with PVC foil.
- Soak up excess liquid with filter paper, stack slides with suitable filter pads and dry overnight at 60 °C under slight pressure in the slide press.
- Only open the press after it has cooled down. Carefully remove the cover foil from the cold slide.
- Further processing of the sections with histological or immunohistochemical staining methods (see following pages).

Recommended laboratory equipment for the application of the Technovit 9100 system

- Chromatography column
- AL_2O_3 (active, basic, 90)
- adjustable refrigerator
- adjustable freezer
- Glass desiccator
- Vacuum pump
- Set of volumetric flasks
- suitable embedding forms
- appropriate microtome
- Cutting-thin-ground system from EXAKT
- Magnetic stirrers

Technical data	
Colour	transparent
density = specific weight g/cm3 (DIN 53479)	1,07
Refractive index monomer polymer	 1,4175 1,4720
Storage temperature	max. 25 °C

Accessories and supplements



Histoform N:
Special embedding moulds made of Teflon with stainless steel bottom for heat evacuation.
With 4 cover plates
4 trays
Dimensions: W x H x D: about 12 x 20 x 10 mm.

Embedding moulds especially for Technovit® 9100
Polyethylene embedding moulds 25 mm & „boat" insert for the round sample holder on the rotary microtome.
Other sizes available:
15 - 30 - 40 - 50 mm (cylinder only)

Kisol foils + filter pads
For separating slides in the slide press.

Pressure roller
For pressing on and better adhesion of the sections on coated slides

Slide press
For pressing the sections onto coated slides.

Chromatography column
For de-stabilizing the base solution for immunohistochemistry.

Set of measuring pistons
For preparing the infiltration and stock solutions.

Poly-L-Lysin-solution
For coating slides for adhesion of T9100 cuts

Primer for the pretreatment of slides
For better adhesion of grindings on glass slides

Light-polymerizing adhesive
For gluing sections onto glass slides

Deplaticizing solutions (MEA, acetone)
For de-plasticising the sections for histological staining.

Embedding in Technovit 9100

Manufacturer: Kulzer GmbH, **Resin type:** (Methylmetacrylat)
Suitability: hard tissue, bones, teeth, biopsies; **fixation:** Ethanol, formalin, PFA, dry samples

Infiltration steps	Duration	Process
Times apply to sample sizes of approx. 5 mm => double times for each additional 5 mm³		
Ethanol 70 %	1 - 3 h	Dehydration, with vacuum
Ethanol 80 %	1 - 3 h	Dehydration, with vacuum
Ethanol 90 %	1 - 3 h	Dehydration
Ethanol 96 %	2 - 4 h	Dehydration
Ethanol 96 %	2 - 4 h	Dehydration
Isopropanol 99,8 % / Ethanol 99,8 %	2 - 4 h	Dehydration
Isopropanol 99,8 % / Ethanol 99,8 %	2 - 4 h	Dehydration
Isopropanol 99,8 % / Ethanol 99,8 %	2 - 4 h	Dehydration
Xylene	3 - 6 h	Dehydration
Xylene	3 - 6 h	Dehydration
Pre-infiltration solution I • 200 ml Basic solution (Component 1) • 200 ml Xylene — **stabilised solution** mix well, store in freezer, keeps for several months	3 - 12 h (under movement!)	Infiltration **briefly drip off or dry with paper when changing**
Pre-infiltration solution 2 • 200 ml Basic solution (Component 1) • 1 g Hardener 1 (Component 3) — **stabilised solution** mix well, store in freezer, keeps for several months	3 - 12 h (under movement!)	Infiltration **briefly drip off or dry with paper when changing**
Pre-infiltration solution 3 *(mandatory for IHC)* • 200 ml Basic solution (Component 1) • 1 g Hardener 1 (Component 3) — For immunohistology: **destabilised** solution mix well, store in freezer, only 4 weeks shelf life	3 - 12 h (under movement! in the refrigerator)	Infiltration **briefly drip off or dry with paper when changing**
Infitration solution • 150 ml Basic solution (Component 1) • 20 g PMMA-Powder (Component 2) — For immunohistology: **destabilised** solution Stir well in 250 ml volumetric flask until solution becomes clear • 2 g Hardener 1 (Component 3) — 1 g if destabilised solution is used Add and continue stirring • 100 ml Basic solution (Component 1) — For immunohistology: **destabilised** solution Add up to 250 ml level in the measuring flask and continue stirring	4 - 24 h (under movement! in the refrigerator)	Infiltration **briefly drip off or dry with paper when changing**

Embedding in Technovit 9100 (continued)

Manufacturer: Kulzer GmbH, **Resin type:** (Methylmetacrylat)
Suitability: hard tissue, bones, teeth, biopsies; **fixation:** Ethanol, formalin, PFA, dry samples

Infiltration steps	Duration	Process
Pouring and curing:		
Stock solution A • 300 ml Basic solution (Component 1) — ***for IHC: destabilised solution*** • 80 g PMMA-Powder (Component 2) Stir well in a 500 ml volumetric flask until the solution becomes clear • 4 g Hardener 1 (Component 3) — ***for IHC: destabilised solution => only 3 g Hardener 1*** and continue stirring until completely dissolved • **about 200 ml** Basic solution (Comp. 1) — ***for IHC: destabilised solution*** add up to 500 ml mark in the measuring flask and stir for 1 h Store in the freezer at a minimum of -18 °C (portion if necessary) **Stock solution B** • 30 ml Basic solution (Component 1) — ***for IHC: destabilised solution*** • 4 ml Hardener 2 (Component 4) • 2 ml Regler (Component 5) mix well in 50 ml volumetric flask • **about 20 ml** Basic solution (Comp. 1) — ***for IHC: destabilised solution*** add up to 50 ml mark in the measuring flask and stir for at least 30 min. Store in the freezer at a minimum of -18 °C **Working solution / embedding solution:** • 90 ml Stock solution A • 10 ml Stock solution B — mix well with glass rod or on stirrer mix well, use immediately and pour in sample	***couple of hours or days, depending on Sample size:*** ***< 15 ml: Freezer: -2 bis -4 °C*** ***> 15 ml: Freezer: -5 bis -10 °C*** ***> 25 ml: Freezer: -18 °C***	Place sample in mould, fill up until upper top and incubate in the desiccator at **200 mbar about 5-10 min**, if possible evacuate under cool temperature. (Release vacuum repeatedly, reevacuate, cooling packs in desiccator helpful) Subsequently seal hermetically
Blocking (if no round sample holder is available)		
Technovit 3040: • 3 spoons Technovit 3040 yellow • 1 spoon Technivit Universal-Liquid — Mix to a viscous mass in a paper cup apply immediately, cures within a few minutes!	few minutes	Place on suitable sample holders
cutting, sawing, grinding		
Inner diameter saw: • Cutting thickness: to at least 20-30 µm • Sawing with water jet **Glue on slide:** • UltraKitt • Entellan	**Hard-cut microtome:** • Cutting thickness: 1 - 10 µm • Sectioning with 30% ethanol **Glue on slide:** • strech with 50% ethanol on ponal coated slides • Covering with Kisol foil, 24 h in press at 60 °C	**Ultra Milling Machine:** • Milling block **Glue on slide:** • Supergluer **Ultra Milling Machine:** • mill the other side
Duration of the entire processing: approx. 2 - 8 days (depending on sample size)		

Staining of Technovit 9100

Technovit 9100 sections and grindings can be stained with all classical histological staining methods. Thin sections can also be processed with immunohistochemical detection methods. However, Technovit 9100 requires prior deplastination. A distinction is made between hard-cut sections and cut & ground sections.

Preparation

The sections are deplastinised for preparation. MEA, MEK, DMSO, acetone, benzene (toluene), carbon tetrachloride, xylene or amyl acetate, for example, can be used. Increased caution is required, as many of these substances are toxic and harmful to health. The deplastination is necessary for histological staining as well as for immunohistochemical detection methods.

Deplastination for sections	
Xylol	2 x 20 min
MEA	2 x 20 min
Acetone	2 x 5 min
Aqua dest..	
Deplastination for cut & ground sections	
Xylol	2 x 10 min
MEA	2 x 2 h
Acetone	2 x 10 s
Aqua dest.	

Application

After deplastination, the samples are placed directly in aqua dest. and histological staining is performed according to protocol. Depending on the type of sample, there may be differences in the intensity of the staining. Therefore the staining times have to be determined empirically depending on the sample type and the desired result.

Exemplary staining protocol Movat

Deplastination

Step 1: Acetic acid 3%	30 s
Step 2: Alcian blue 1%	30 min
Step 3: washing	8 min
Step 4: Haematox-Verhöff	8 min
Step 5: EisenIIIChlorid 1%	1 min
Step 6: washing	7 min
Step 7: Brilliant Crocein	6 min
Step 8: Acetic acid 1%	30 s
Step 9: Phosphotungstic acid	10 min
Step 10: VE waterr	1 min
Step 11: Ethanol 99%	3 min
Step 12: Ehtanol 99%	3 min
Step 13: Saffron du Gatinais	8 min
Step 14: Ethanol 99%	90 s
Step 15: Ethanol 99%	2 min
Step 16: Isopropanol	5 min
Step 17: Xylol	5 min
Step 18: Xylol	5 min

Immunohistochemistry

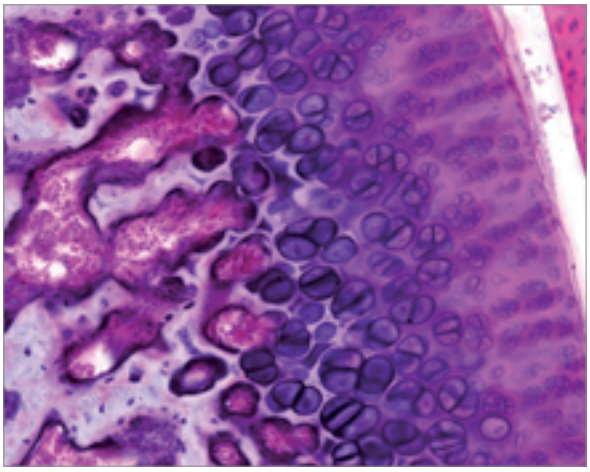
Technovit 9100 sections can also be treated with immunohistochemical detection methods. For this purpose, the samples are first deplastisized and then treated according to the usual procedure. Enzymatic (e.g. with trypsin) pretreatment is followed by incubation with primary and secondary antibodies. This is followed by counterstaining, for example with hematoxylin.

Post-treatment and coverslipping:

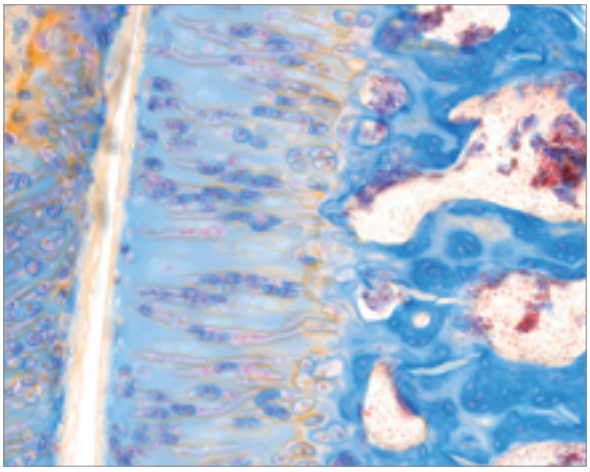
Stained sections are dehydrated to xylene via an ascending alcohol series and can be coverslipped like paraffin sections with CV-mount in the automatic coverslipping machine. Further post-treatment is not necessary.

Catalog no.	Description
12156	Hematoxylin & Eosin (H&E)
12043	Masson Goldner
12061	Movat Pentachrom after Verhöff

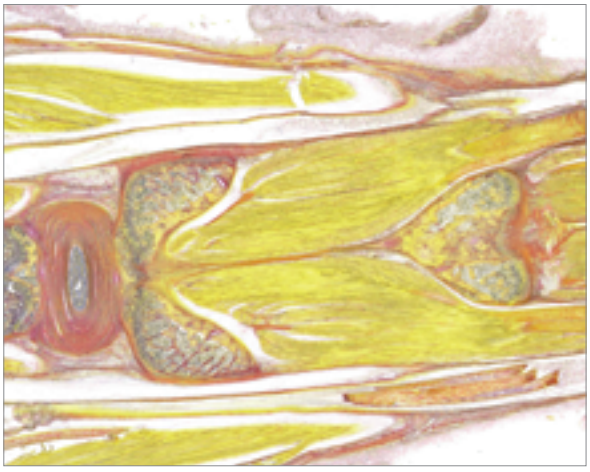
Stainings examples of Technovit 9100



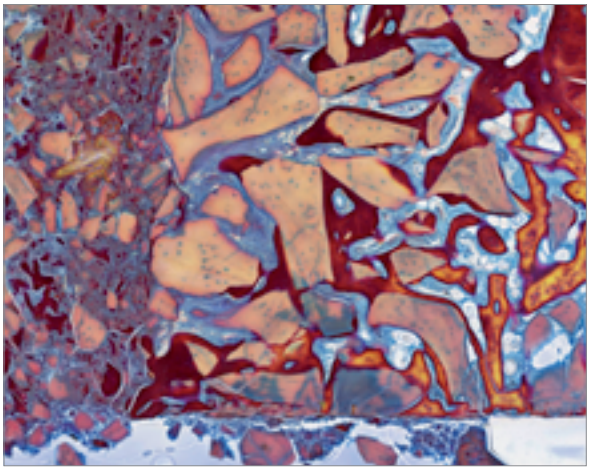
Rat, Knee, Hematoxylin & Eosin, Catalog no.: 12156



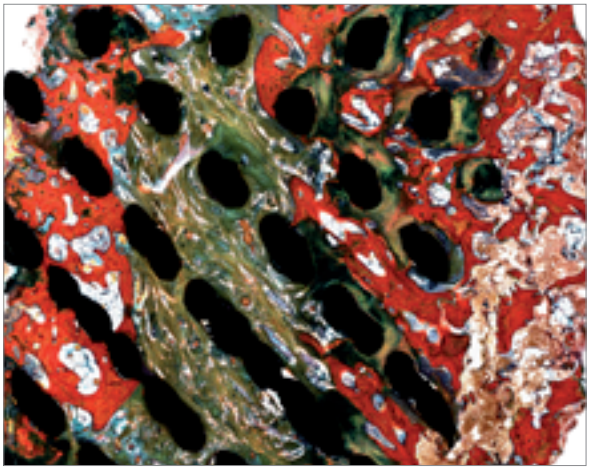
Rat, Elbow, Azan after Geidies, Catalog no.: 12082



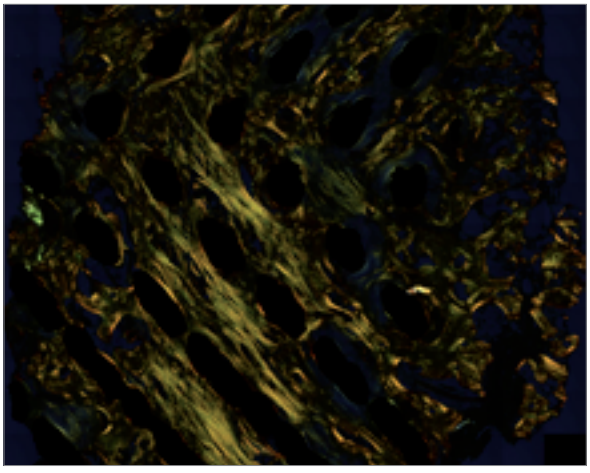
Rat, Spine, Pikrosirius Red, Catalog no.: 13425



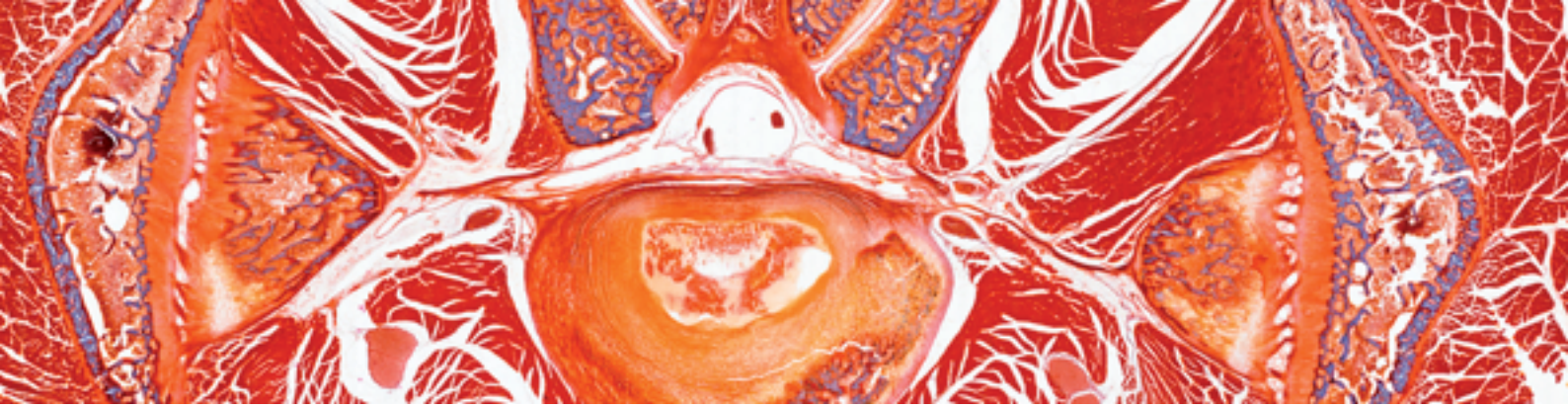
Human, Bone, Herovici, Catalog no.: 18432; with kind permission by Geistlich Pharma AG



Human, Explantate, Movat Pentachrom after Verhöff, Catalog no.: 12061; with kind permission by Emerging Implant Technologies



Human, Explantate, Pikrosirius Red (Polarized), Catalog no.: 13425; with kind permission by Emerging Implant Technologies



Technovit® 7200 VLC

Light-curing resin for thin section production

Technovit 7200 VLC is an innovative and easy-to-use resin system for the production of thin sections of non-cuttable tissue (e.g. teeth, bones, implants), materials or other hard samples (metals, rocks, resins). Technovit 7200 VLC is a light-curing one-component resin that does not depend on aggressive UV light sources. Instead, it is stimulated to polymerise with simple white and blue light and polymerises slowly and evenly.

Properties

Technovit 7200 VLC was specially developed for the cutting-grinding technique for histological examination of mineralised tissue (teeth, bones). It completely penetrates the hard tissue, which is why decalcification of the tissue is not necessary with this technique. Resins such as composite tooth fillings or bone cements are not attacked by Technovit 7200 VLC. There is also unrestricted compatibility with all types of ceramic, metal and resin implants or specimens. The Technovit 7200 system is therefore ideally suited for histopathological examinations in research and routine.

The polymerisation process is initiated by a combination of blue and white light and usually takes place within a few hours. If the indicated irradiation times are observed, the temperature of 40 °C is not exceeded, in contrast to many UV-curing embedding systems.

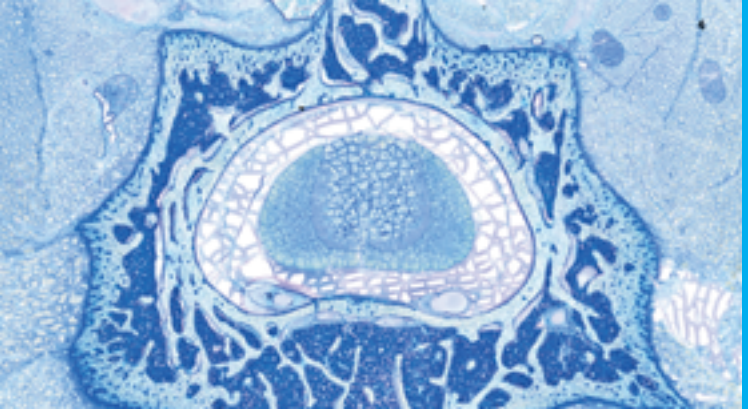
Finished polymerized specimens have a high mechanical strength, so that it is possible to produce cut & ground sections down to a thickness of a few micrometers. The cutting and grinding systems from EXAKT are particularly suitable for this purpose. EXAKT also offers special embedding, bonding and polymerisation devices for the Technovit 7200 VLC system (light bonding press, light polymerisation unit, drying unit).

Thin sections of Technovit 7200 VLC embedded samples can be processed with many classical histological staining techniques. MORPHISTO offers a wide range of staining kits and individual solutions that are especially suitable for Technovit embedded sections.

Technovit 7200 VLC fulfils all requirements for reliable specimen preparation for the production of histological samples in anatomy, pathology and orthopaedics, trauma surgery, dentistry, oral and maxillofacial surgery, materials science, materials testing, etc..

The advantages at a glance

- no bubbles
- Applicable in tissue processors
- no stress cracks
- Polymerisation stable up to high degrees of hardness
- no thermal stress on tissue
- Light polymerisation in maximum 6 h
- Temperature maximum 40 °C during polymerisation
- divers histological staining possible
- easy handling of device and material
- unlimited shelf life of the samples



Technovit 7200 VLC was specially designed for the cutting-grinding technique.

The components

Technovit 7200 VLC

Light-curing one-component resin based on methacrylate for embedding and subsequent thin sectioning in medicine and dentistry.

Technovit 7210 VLC

Light-curing one-component precision adhesive based on methacrylate for precision bonding of the cured samples on an acrylic glass slide.

Technovit 7230 VLC

Light-curing one-component adhesive based on methacrylate for fixing the tissue sample in the embedding moulds.

Product data

Catalog no.	Description	Content
17571.01000	Technovit 7200 VLC	1000 ml Embedding medium
17571.00030	Technovit 7210 VLC	2 x 15 ml Precision adhesive
17571.00100	Technovit 7230 VLC	100 ml Fixation adhesive
18111.K3000	Technovit 4000 kit	750 g Powder 250 ml Syrup I 500 ml Syrup II
	Embedding mould I	50 pieces
	Embedding mould II	50 pieces
18111.F1500	Powder	1500 g
18111.01000	Syrup I	1000 ml
18111.00500	Syrup II	500 ml

Technical data	
Colour	transparent
density = specific weight g/cm3 (DIN 53479)	1,07
Refractive index monomer polymer	 1,4175 1,4720
Storage temperature	max. 25 °C
Shelf life	2 years

The processing

Fixation

Organic tissue samples should be fixed before embedding in Technovit 7200 VLC. All common fixation media such as formalin, PFA, alcohol and others are suitable. The use of fixatives based on picric acid or mercury is possible in principle, but should be tested. In the case of mercury fixation, iodine treatment must take place before the infiltration process. Picric acid must be completely removed by an alcohol series in order not to impair the polymerisation process.

Dewatering / dehydration

The dewatering of the tissue is achieved by an ascending alcohol series. The usual infiltration times apply here. Movement (agitation) is generally advantageous. Experience has shown that the more massive the hard tissues are, the longer the infiltration times of the alcohol levels have to be increased significantly.

Infiltration

The actual infiltration is introduced with a mixture of alcohol/Technovit 7200 VLC = 1 : 1. This is followed by two further intermedium steps with a mixing ratio of 1 : 2 and 1 : 3 and then infiltration is carried out with at least two stages of pure Technovit solution.
If the samples are very solid, or if it can be assumed that the light will not reach the deeper layers of the sample, 1 g of the hardener (BPO) should be added to each pure Technovit solution.

Instead of alcohol, a mixture of glycol methacrylate/Technovit 7200 can also be used as an intermedium. Here it is important to gather your own experience in order to establish economical infiltration processes.

In the case of automated dehydration and infiltration with agitation and vacuum, dehydration times of 3 hours per stage and infiltration times of 8 hours per stage are sufficient for 2 to 3 mm thick tissue samples. For dehydration and infiltration without agitation and vacuum, the times per stage must be approximately quadrupled. The infiltration must be carried out in opaque vessels or automatic machines, as otherwise polymerisation is induced by light incidence.

Dehydration and infiltration via alcohol (Starting point: formalin sample from 70% ethanol)

Phase	Solution	Concentration	Infiltration time	
			Bones	Teeth
Dehydration 1	Ethanol	70 %	1- 2 h	4- 6 h
Dehydration 2	Ethanol	80 %	1- 2 h	4- 6 h
Dehydration 3	Ethanol	90 %	1- 2 h	4- 6 h
Dehydration 4	Ethanol	96 %	1- 2 h	4- 6 h
Dehydration 5	Ethanol	99 %.	1- 2 h	4- 6 h
Dehydration 6	Ethanol	99 %.	1- 2 h	4- 6 h
Infiltration I	Ethanol / Technovit	1 : 1	8 - 24 h	2 - 6 days
Infiltration II	Ethanol / Technovit	1 : 2	8 - 24 h	2 - 6 days
Infiltration III	Ethanol / Technovit	1 : 3	8 - 24 h	2 - 6 days
Infiltration	Technovit 7200	pure	8 - 24 h	2 - 6 days
Infiltration	Technovit 7200	pure	8 - 24 h	2 - 6 days

Embedding

For plane-parallel embedding, the prepared, infiltrated tissue sample is placed in the corresponding embedding form I or II on a previously applied drop of Technovit 7230 VLC fixing adhesive and lightly pressed on. Care must be taken to ensure that the surface to be examined is placed at the bottom. Then pour Technovit 7200 VLC over the tissue sample so that the tissue does not float and no air bubbles are trapped.

Polymerisation

The polymerisation of the embedded sample is performed in a blue light unit. The polymerisation is carried out in two steps.

1st step:

During the first polymerisation stage, the embedding medium is polymerized to a large extent. This is carried out at low light intensity (white light) so that the polymerisation temperature does not exceed 40 °C and no stress cracks occur.

2nd step:
In the second polymerisation stage, the resin infiltrated tissue is completely polymerized at high light intensity (blue light). The total polymerisation time is 6 hours maximum. The polymerisation temperature does not exceed 40 °C with a maximum of 40 ml embedding medium and at a room temperature of 23 °C.

Further processing after curing

After the complete polymerisation the blocks can be prepared for further processing by the cutting-grinding technique. The usual procedure here is:

- plane-parallel gluing of the block to a slide or sample holder
- plane-parallel grinding of the sample side or plane-parallel trimming with a diamond band saw, inner hole saw or saw
- Polishing the sample side
- Adhering to a microscope slider
- Cutting off a section as thin as possible with a diamond band saw, inner diameter saw or saw
- Grinding of the cut-off section to the desired thickness and subsequent polishing
- Staining with suitable histological staining techniques
- Coverslipping with a protective covering glazes
- Microscopy and evaluation

In the process of cutting-grinding technology, only the points concerning Technovit components and accessories are mentioned here. The method of cutting and grinding can be found in the respective manuals and training programmes of the equipment manufacturers.

Technovit 4000

In order to get the polymerised blocks out of the plastic moulds again, or to stick them onto suitable holders (e.g. microscope slides) for further processing in cutting-grinding systems, a non-shrinking resin, Technovit 4000, is used.
Technovit® 4000 is a fast-curing cold-curing 3-component resin based on modified polyester in the form of powder and syrup I and II. The primary application is the encapsulation of materials for subsequent grinding and polishing of surfaces.

Material properties

Technovit 4000 is particularly characterised by low polymerisation shrinkage and optimum edge seal. The embedding of geometrically demanding samples is guaranteed by the good flow behaviour of Technovit 4000. Due to the very good adhesion to metal, Technovit 4000 guarantees a very low-gap embedding. These properties are particularly important for all specimens where a good edge sharpness is important.

Procedure

After complete polymerisation, a Technovit 4000 paste is stirred, applied to the block and then the slide or block holder is pressed on.
The mixing ratio for the three components is
2 : 2 : 1 (powder : syrup I : syrup II), whereby syrup I and II are first mixed together and then the powder is stirred in. Technovit 4000 can be poured for approx. 4 minutes after mixing, the curing time is approx. 8 minutes.

Depending on the room temperature, Technovit 4000 is cured in 15 - 20 minutes and further processing by diamond band saw can begin.

Blocking out, further processing, archiving

Blocking out and gluing on

The polymerized tissue block removed from the embedding mould is placed on the base plate of the vacuum adhesive press (EXAKT construction) in the same direction as it was placed in the embedding mould. Technovit 4000 is applied to the surface of the polymerised tissue block. The cover plate with the vacuum held microscope slide of the vacuum adhesive press is lowered until it comes into contact with Technovit 4000 and locked with a screw. After completion of the polymerisation, the block is inserted into the micro-grinding system and excess Technovit 7200 VLC is removed from the surface to be examined.

Cutting and grinding

Technovit 7200 allows the production of very thin grinding sections due to its excellent material properties and stability. Depending on the nature of the embedded sample, thicknesses of 70 - 100 µm are possible. The actually desired grinding thickness (usually 5 - 15 µm) is only achieved in the second step, the grinding process. Suitable instruments for these techniques are:

- EXAKT C300 diamond band saw
- EXAKT C310 diamond band saw
- Leica inner diameter saw SP 1600
- EXAKT grinding machine 400 CS
- Other grinding machines with absolute thickness measurement

Gluing on slides

Technovit 7200 sections can be glued onto commercially available glass slides or suitable acrylic glass slides from EXAKT. The resin Technovit 7210 VLC is used here. The photopolymerisation of the adhesive is carried out in the precision adhesive press from EXAKT and is completed after 15 minutes of curing time.

Staining of thin sections

Thin sections of Technovit 7200 can be processed with many satins using classical histological methods (see page 36f). In contrast to Technovit 9100, Technovit 7200 resin cannot be removed, so there are no deplastination procedures. Therefore, staining, as with Technovit 7100, can only be carried out on the surfaces of the tissue, although certain pre-treatment of the specimens is necessary here. MORPHISTO has developed a number of suitable procedures and offers a wide range of suitable staining kits for Technovit 7200.

Storage and archiving of the samples

Samples embedded in Technovit 7200 have an unlimited shelf life at room temperature. Too strong temperature fluctuations should be avoided as well as too high air humidity. The shelf life applies to blocks as well as to cut & ground sections. The latter are best stored horizontally in suitable slide boxes.

Embedding in Technovit 7200

Manufacturer: Heraeus Kulzer, **Resin type:** GMA (Glycolmethacrylat)
Suitability: hard tissue, **fixation:** formalin, PFA, dry samples

Infiltration steps	Duration	Process
Times apply to sample sizes of approx. 3-5 mm => double times for each additional 5 mm³		
Ethanol 70 %	1 - 3 h	Dehydration
Ethanol 80 %	1 - 3 h	Dehydration
Ethanol 90 %	1 - 3 h	Dehydration
Ethanol 96 %	2 - 4 h	Dehydration
Ethanol 96 %	2 - 4 h	Dehydration
Ethanol 100 %	2 - 4 h	Dehydration
Ethanol 100 %	2 - 4 h	Dehydration
Intermedium 1: Technovit ethanol mixture • 30 ml Basic solution Technovit 7200 • 70 ml Ethanol 96 % alternatively GMA possible	8 - 48 h (under movement)	Infiltration
Intermedium 2: Technovit ethanol mixture • 50 ml Basic solution Technovit 7200 • 50 ml Ethanol 96 % alternatively GMA possible	8 - 48 h (under movement)	Infiltration
Intermedium 3: Technovit ethanol mixture • 70 ml Basic solution Technovit 7200 • 30 ml Ethanol 96 % alternatively GMA possible	8 - 48 h (under movement)	Infiltration
Infitration solution • Basic solution Technovit 7200 if nec. 1 g BPO per 100 ml	8 - 48 h (under movement)	Infiltration
Infitration solution • Basic solution Technovit 7200 if nec. 1 g BPO per 100 ml	8 - 48 h (under movement)	Infiltration
Pouring and curing		
Pouring solution / embedding solution **normal to transparent samples:** • Basic solution Technovit 7200 fresh solution ! **massive very dark samples:** • 100 ml Basic solution Technovit 7200 • 1,0 g Hardener 1 (= 1 package) fresh solution !	6 h White light 6 h Blue light 6 h Post-curing in daylight	Embed the samples into transparent plastic moulds, if necessary fix with Technovit 7230 VLC Polymerisation with blue light and white light
cutting, sawing, grinding		
Diamond band saw: • cut & ground section **Rotationssäge:** • cut & ground section	**Hard-cut microtome:** • not cuttable • **Glue on slide:** • VLC 7210	**Ultra Milling Machine:** • Block und sections millable
Duration of infiltration: approx. 4 - 12 days (depending on sample size)		

Staining of Technovit 7200

Technovit 7200 thin sections can be stained according to many known histological staining methods, for example to better differentiate tissue.
As with Technovit 7100 and 8100 sections, Technovit 7200 cannot be removed from the samples. Therefore, when staining, it must be considered that not the complete section is stained, but only the upper layer that comes into direct contact with the staining solution. For T7200 sections, however, pre-treatment is necessary to ensure a stainable surface. In addition, longer incubation times are usually necessary to achieve an equivalent staining result.

Preparation

Technovit 7200 sections are etched before staining to create a surface that absorbs the colour. For this purpose, 0.1% formic acid, 10% hydrogen peroxide or 10% citric acid can be used. The sections are incubated for about 30 minutes in the respective solution before the staining is performed. Staining should take place immediately after etching of the samples.

Procedure

The histological staining is performed as usual. First, fine structures such as cell nuclei are stained. This is followed by staining of cell plasma and finally fibres and basic substance. The staining times vary according to the desired result and sample type and must therefore be determined empirically with each new sample type. It should also be noted that the staining result may be different from that of paraffin-embedded samples.

Exemplary staining protocol Movat

Etching

Step 1: Acetic acid 3%	30 s
Step 2: Alcian blue 1%	30 min
Step 3: watering	4 min
Step 4: Hematoxylin-Verhöff	8 min
Step 5: Iron III chloride 1%	1 min
Step 6: watering	7 min
Step 7: Brilliant Crocein	6 min
Step 8: Acetic acid 1%	30 s
Step 9: Phosphotungstic acid	10 min
Step 10: VE Water	1 min
Step 11: Ethanol 99%	1 min
Step 12: Ethanol 99%	1 min
Step 13: Saffron du Gatinais	2 min
Step 14: Ethanol 99%	1 min
Step 15: Ethanol 99%	1 min

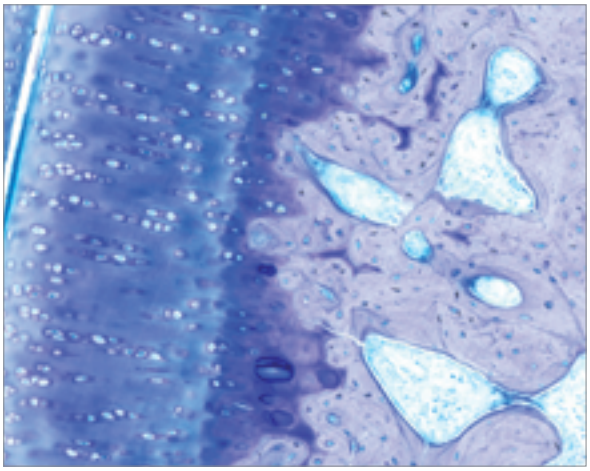
Post-treatment and coverslipping

Stained sections are not dehydrated over an ascending alcohol series, but only washed briefly in 96% ethanol. The slides are then dried and manually coverslipped with Technovit 7200. For curing the slides are treated for 30 minutes under white light and then for 30 minutes under blue light.

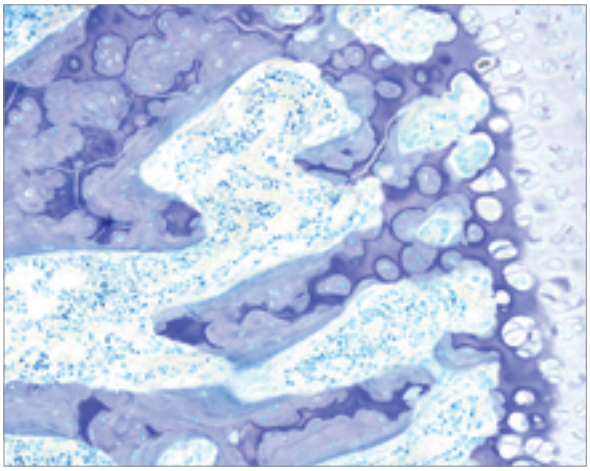
Comparison Technovit 9100 and 7200 before/at/after staining

	T9100	T7200
before staining	**deplasticizing**	Etching
	no dewaxing	
at staining	directly in aqua dest. or staining solution	
after staining	- ascending alcohol series to Xylene - coverslipping with CV Mount/ Automaton	- only briefly in 96% Ethanol - let dry - Manual coverslipping with T7200 - 30 min. White Light - 30 min. Blue Light

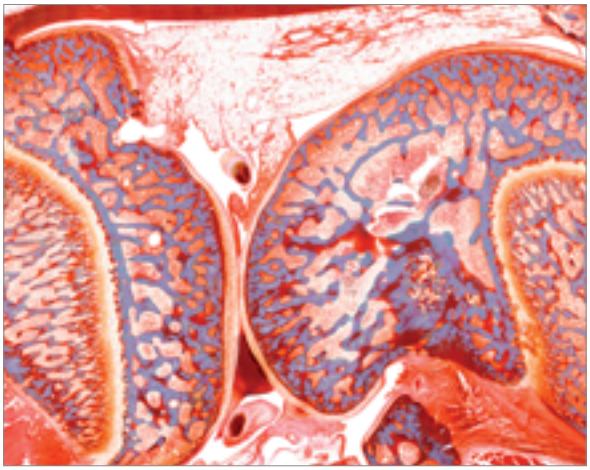
Stainings examples of Technovit 7200



Rat, Knee, Toluidine blue, Catalog no.: 13469

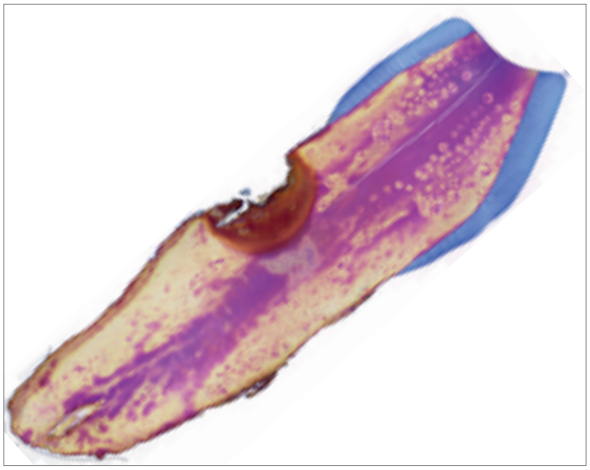


Rat, Knee, Toluidine blue, Catalog no.: 13469

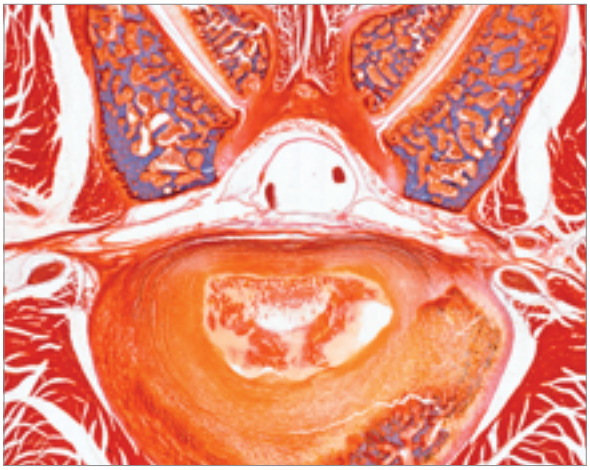


Rat, Knee, Masson Goldner Light Green,
Catalog no.: 12043

Rat, Lower jaw, Masson Goldner light green,
Catalog no.: 12043



Human, Tooth, Herovici, Catalog no.: 18432



Rat, Spine, Masson Goldner aniline blue,
Catalog no.: 12043

Accessories and additional supplies

Embedding moulds I and II
For plane-parallel embedding of infiltrated tissue samples

Technovit 4000
Fast curing 2-component resin on methyl methacrylate base (MMA). For blocking and fixing embedded resin specimens with the Histobloc or for base embedding.

Mixing spatula
Wood spatula for mixing Technovit 3040.

Mixing cup
Paper cup for mixing Technovit 3040.

Resin microscope slide 76 x 25
Technovit 7200 sections can be applied to acrylic glass or glass slides. These acrylic glass slides are suitable for smaller samples.

Resin microscope slide 100 x 50
Larger microscope slides made of acrylic glass for adhering of Technovit 7200 sections.

Glass slides 76 x 24
Commercially available glass slides for adhering of Technovit 7200 sections.

EXAKT equipment

Diamond band saw
EXACT 300 CP
The diamond band saw is used to cut highly sensitive tissue and tissue combinations for the histological analysis of muscle tissue, bone and especially bone with implants or screws. Both fresh and embedded samples can be processed.

Micro grinder
EXACT 400 CS
Grinding machine for the production of consistently plane-parallel thin sections of up to 20 µm with excellent surface quality.

Splicer
EXACT 401
Gluing press for precise and plane-parallel application of thin sections of up to 10 µm thickness on the slide.

Light adhesive press
EXAKT 402
Device specially developed for Technovit 7200 embedding, which controls the curing time, degree of curing and temperature development and ensures optimum polymerisation results.

Drainage & infiltration device
EXACT 510
Device developed for the infiltration and dehydration of samples, which can be operated with up to 6 infiltration stages simultaneously, protected from light. A movement of the device ensures optimal dehydration and penetration of the resin. If required, also under vacuum to eliminate any risk of artifacts.

Light polymerisation unit
EXACT 520
Gluing press which, combined with the adhesive Technovit 7210, achieves an optimum layer thickness and perfect results.

Drying unit
EXACT 530
Device developed for the post-processing of samples, which can remove residual water from the sample by means of vacuum and controlled heat or post-cure the sample by blue light.

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