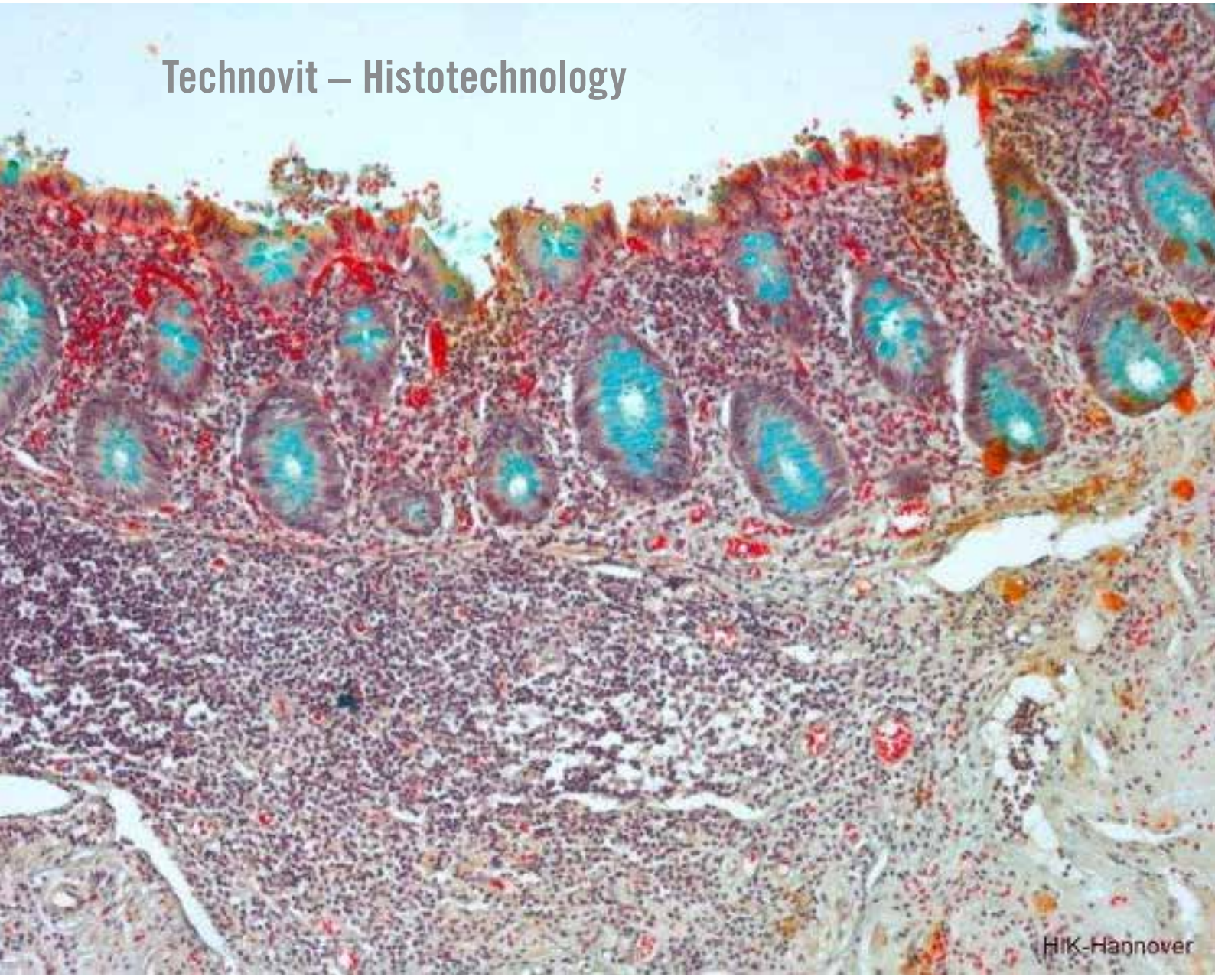
Heraeus Kulzer
Mitsui Chemicals Group

Technovit – Histotechnology



Polymerisation Systems for Histological Applications

Technovit
Polymerisation Systems for Histology

The systems developed by Heraeus Kulzer in collaboration with renowned scientists and institutes to embed tissue in plastic material for histological studies have proven themselves in practice for decades. They are successfully used for diagnostic and research purposes in medicine, veterinary medicine and botany. They are also successfully used in cutting technology for industrial applications and biomaterials.

The systems fulfil important requirements such as embedding at low temperatures, thin and semi-thin section techniques and optimal division and abrasion properties. The sections can be easily stretched, and under the light microscope the stained preparations show excellent morphology.

The scientific and economic conditions for histological studies of tissues are significantly improved with Heraeus Kulzer's histology technology

- Simple, rational handling, as all components are compatible with one another
- Due to the special material properties, the standard stain methods, enzyme chemistry and immunohisto-chemistry used in histological laboratories can be used, including in-situ hybridisation

Why plastic?
In contrast to all other embedding materials used in light microscopy in histological technology, uniform thin and semi-thin sections can be made after plastic has been embedded. In the process morphological details remain excellent. The mineralised and cellular structures can be better determined in undecalcified specimens embedded in plastic. The mineral matrix and the cartilagenous and ligament tissues are very well maintained.

The results of enzymatic immunohistochemical studies and in-situ hybridisation show more sensitive and specific activity because all Technovit plastics harden at low temperatures and below freezing temperatures due to the special composition of the polymerisation systems.

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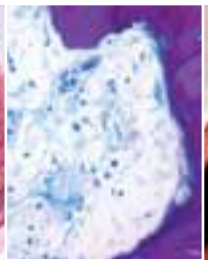

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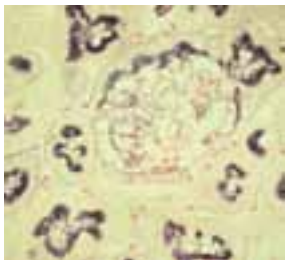
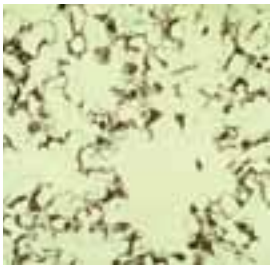
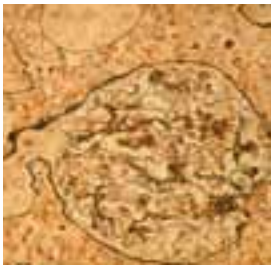
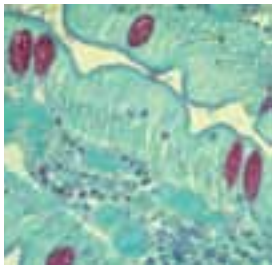
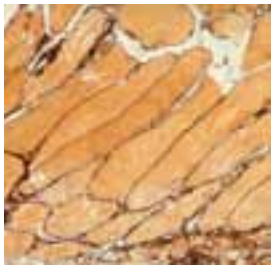


Technovit 7100
Sections for histological and industrial application

Technovit 7100 is a plastic embedding system based on HEMA* (2-hydroxyethyl methacrylate). The hydrophillic resin is used in medicine, botany, zoology and in the industry for embedding tissues for light microscope studies. The sections can be used for histological staining and enzyme detection.

Technovit 7100 transparently polymerises. Uniform thin sections can be made out of the blocks with the rotation microtome. It is not necessary and also not possible to elute the plastic out of the block and the section.

Material properties
The chemical polymerisation of Technovit 7100 is initiated using a barbituric acid derivative in combination with chloride ions and benzoyl peroxide. The catalyst system does not have any aromatic amines compared to traditional systems.

Overview of the benefits
- Uncomplicated handling
- Reproducibility and reliability of the embedding due to the constant, documented quality controls of the individual components
- Low polymerisation temperature due to Teflon embedding forms
- Uniform hardening of the block, thus uniform and thinnest possible sections
- Low shrinkage artefacts, thus excellent tissue morphology
- In addition to routine staining, enzyme detection is also possible
- Less toxic due to catalyst made of barbituric acid
- Polymerisation at room temperature (20°C)
- Airtight sealing not necessary while hardening
- Decalcification not necessary for haematological illiac crest biopsies

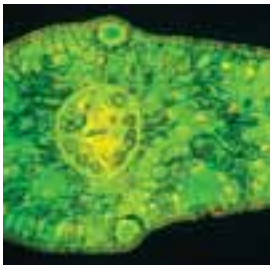
Application
Prepare Technovit 7100 in accordance with the step-by-step instructions. First place the fixated and dehydrated specimens in the pre-infiltration solution and then in the infiltration solution. Vacuum intervals and/or agitation during the individual steps accelerate the embedding process.
Specimens for industrial application are placed directly in the infiltration solution or hardened without pre-treatment.

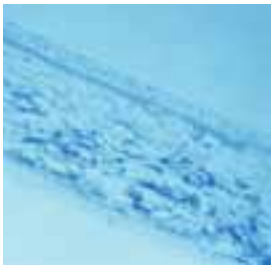
Polymerisation
Prepare the polymerisation mixture according to the instructions and then fill the embedding cavities with it. Immediately position the infiltrated specimens therein. Polymerisation occurs at room temperature.

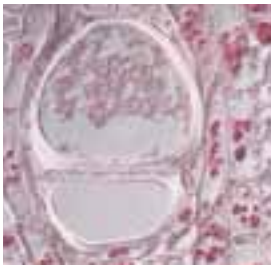
Polymerisation temperature in:
- Histoform S 32 °C – 38 °C
- Histoform Q 40 °C – 45 °C

Block after hardening (approx. 1½ hrs.) with Histobloc® and Technovit 3040 (see also page 15).

* HEMA is also known as GMA (Glycolmethacrylate)







Technovit 7100
can be used in many
fields. Application:
organic preparations,
plastics, films & paper,
fibres, and much more...

Other fields of application
Technovit 7100 is THE choice material for anything that pertains to making sections out of the embedded materials. The Technovit 7100 - which was originally designed for histology - has proven itself in the industry for years due to its universal applicability.

For example, the fields of application include embedding and sections made out of:
- Plastics
- Films
- Paper
- Textiles
- Organic preparations
- Fibres

Material properties
In cases where material specimens are cut and examined with a light microscope, embedding Technovit 7100 facilitates sections in the u-area.
The specimens can be economically cut in high quality with the Technovit Histoblade, a disposable blade coordinated with this system.

These types of preparations are only slightly different from medicinal specimens.
In many cases it is not necessary to place it in the preparation solution, as is the case with films and plastics.

Instructions for embedding non-medicinal tissue specimens
- Infiltration in the preparation solution with vacuum is recommended for paper and textiles
- Biological preparations are medically handled (fixed, dehydrated and subsequently infiltrated)
- Materials that are less temperature-sensitive can also be embedded as large specimens (Histoform S and Q, or similar)

Product data

Item No.	Designation	Quantity
64709003	Technovit 7100 Combipack	1 x 500 ml basic solution 5 x 1g hardener 1 1 x 40 ml hardener 2

Technical data

Colour	Transparent
Density = spec. weight g/cm³ (DIN 53479)	1,07
Refractive index	
Monomer	1,4540
Polymer	1,5050
Storage temperature	max. 25 °C
Shelf life	2 years

Step by Step

Depending on the quality of the industrial specimens, e.g. non-porous materials such as films, etc., steps 1-4 can be omitted (fixing to infiltration) Polymerisation can begin directly.

Fixation
Fix and retreat the pieces of tissue as needed.

Dehydration
Dehydration occurs in ethanol or acetone; gradual dehydration is possible with HEMA in buffer (+4°C). Alternating vacuum and agitation are helpful for better penetration during the embedding process. An intermedium is not necessary prior to pre-infiltration.

Pre-infiltration
Prepare the final concentration of the dehydration series in equal parts with basic solution Technovit 7100. Approx. 2 hours at room temperature (20°C), e. g. 50 ml 96% ethanol: 50 ml of Technovit 7100 basic solution.

Infiltration
Making the infiltration solution:
- Technovit 7100 basic solution 100 ml
- + Technovit 7100 hardener 1 1 g (1 bag)

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

Polymerisation
Making the polymerisation solution:
- Mix infiltration medium (unused) 15 ml
- + Technovit 7100 hardener 2 1 ml

for approx. 3–5 Min. mix.
Use standard pipetting aids and disposable container!

Fill the Histoform embedding cavities halfway with the polymerisation solution (disposable pipette), position the prepared specimen therein and fill the form (warning: only the cavity, not the entire recess). Polymerisation occurs either completely (2 hrs.) at room temperature or 1 hour at room temperature and subsequently 1 hour at 37°C in the heating cabinet.

The slightly sticky surface (inhibition layer) can be removed with a lint-free disposable cloth.
Humidity that is too high favours a stronger inhibition layer!

Maximum polymerisation temperature's dependence on ambient temperature

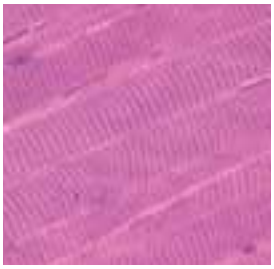
Ambient temperature	Histoform S	Histoform Q
Room temp. approx. 20 °C	32	37
Refrigerator +4 °C	16	23
Refrigerator on ice 0 °C	10	18

Blocking and archiving
The specimen are blocked with Histobloc® and Technovit® 3040 so that they can be removed from the Teflon mould (see page 15).

Processing
Tightly clamp the blocks in the totem cam devices on the rotation microtome. Use the Technovit Histoblade (in combination with the Heraeus blade holder) or hard metal knife (note knife angle) to cut. Dryly remove the section with forceps and place in a bath (aqua dest.)
Place on a clean, grease-free and coated object holder. Let dry before staining for at least 15 minutes or over night at 60°C. Place the deplasticized sections directing in the stain solution. A 1µ-section must be stained longer than a 5µ section for the desired stain intensity.

Overview of how to make a solution

Solution	Ethanol	Basic solution Technovit 7100	hardener 1 Technovit 7100	Infiltration-solution	hardener 2 Techovit 7100	Application temp.
Pre-infiltration	e. g. 50 ml	e. g. 50 ml				Room temperature
Infiltration		100 ml	1 g (1 bag)			Room temperature/ 4 °C
Polymerisation				15 ml	1 ml	Room temperature / 37 °C
				30 ml	1,5 ml	Room temperature /37°C



Sections with Technovit 8100 can be used for more than just histological staining; they can also be used for enzyme chemistry and immunohisto-chemistry.

Technovit 8100
Sections specifically for immunohistochemistry

Technovit 8100 is a HEMA-based plastic-embedding system for studies with light microscopy. It is suitable for embedding all tissues in medicine, zoology and botany. Sections of decalcified or briefly decalcified illiac crest biopsies and implanted biomaterials can be used for more than just histological staining; they can also be used for enzyme chemistry and immunohistochemistry.

Material properties
Technovit 8100 is a combination of a practically odourless plasticizer and a hydrophillic plastic. Technovit 8100 was specifically developed for cold polymerisation (+4°C).

While hardening the embedding form must be hermetically sealed because the polymerisation system is oxygen-sensitive.

Overview of the benefits
- Reproducibility and reliability of the embedding due to the constant, documented quality controls of the individual components
- Low polymerisation temperature of 10°C to 0°C due to the special catalyst system and the Teflon forms
- Uniform hardening of the block, thus uniform and thinnest possible sections
- Low shrinkage artefacts, thus excellent tissue morphology
- Routine staining, enzyme detection and immunohisto-chemistry possible
- Haemotological illiac crest biopsies do not need to be decalcified
- Low toxicity due to special combination of plasticizer and catalyst system

Application
Prepare Technovit 8100 in accordance with the step-by-step instructions. Place the fixated and dehydrated specimens in the infiltration solution. A low temperature and agitation of the specimens is beneficial during the entire embedding process.

Polymerisation
Prepare the polymerisation mixture according to the instructions and then fill the embedding cavities. Position the infiltrated specimens therein. Hermetically cover the cavities with films. Place on a pre-cooled gel plate or thin layer of ice at 4°C to harden.

The films are removed after polymerisation is complete and blocked with Histobloc® and Technovit® 3040.

It is not possible to elute the plastic before staining or reaction.

Product data

Item No.	Designation	Quantity
64709012	Technovit 8100 Combipack	1 x 500 ml basic solution 5 x 0,6 g hardener 1 1 x 30 g hardener 2 1 x 500.pcs. PE films

Technical data

Colour	Transparent
Density = spec. weight g/cm3 (DIN 53479)	1,08
Refractive index	
Monomer	1,4485
Polymer	1,4990
Storage temperatur	max. 25 °C
Shelf life	2 years

The following instructions for fixation and dehydration are not necessarily required. Technovit can also be infiltrated and polymerised after other pre-treatment.

Airtight glass or PE disposable containers (approx. 20 ml) must be used for the entire process!

Tip:
The specimens must be constantly agitated during fixation, dehydration and infiltration!

Fixation
In order to achieve optimal immunohistochemical results, it is recommended to work at 4°C throughout the entire embedding process and to aim for short fixation times. Fix the smallest possible pieces of tissue (1mm thickness) in 2% paraformaldehyde in phosphate buffer pH 7.4 at 4°C for 3-4 hours. Subsequently, retreat for 12 hours (overnight) in phosphate buffer pH 7.4 with an additional 6.8% sucrose at 4°C.

Dehydration
Dehydrate the tissue in cold acetone 100% for at least one hour at 4°C. Change as often as possible in the first minutes until the acetone remains clear.

Infiltration
Making the infiltration solution:
- Technovit 8100 basic solution 100 ml
- + Technovit 8100 hardener 1 1 bag, 0,6 g

Dissolve in a clean, detergent-free PE or glass container and then place at 4°C. When sealed, the infiltration solution is stable for a maximum of four weeks at 4°C.

Transfer the specimen directly from the acetone to the pre-cooled infiltration solution. The specimens remain therein for 6-10 hours at 4°C.

Polymerisation
Making the polymerisation solution:
- Infiltrationsolution, 4 °C 15 ml
- + Technovit 8100 hardener 2, cooled 0,5 ml

Measure with standard pipetting aids and mix well in a PE or glass container. Then, carefully mix the infiltrated specimen in a sealed container for approx. five minutes.

The colour of the polymerisation solution changes first to yellow-green, but after hardening it becomes colourless.

Completely fill the Histoform cavities with a disposable pipette, position the tissue therein and immediately

Overview of how to make the solution

Solution	Ethanol	Basic solution Technovit 8100	Hardener 1 Technovit 8100	Infiltration solution	Hardener 2 Techovit 8100	Application-temp.
Infiltration		100 ml	0,6 (1 bag)			4 °C
Polymerisation				15 ml	0,5 ml	4 °C

cover with transparent PE film. Multiple films can be used for a cavity in order to hermetically seal the cavity. Do not press out bubbles; rather, apply more polymerisation solution and add new film. During polymerisation (at least 3 hours) the embedding form must be placed on a cooling plate or thin layer of ice at 4°C. Do not let the form or specimens come into contact with moisture.

Histoform Q

Material	Room temp. approx. +20 °C	Refrigerator +4 °C	Refrigerator on ice 0 °C
Technovit 8100 30:1	–	69	48
Technovit 8100 35:1	–	52	42
Technovit 8100 40:1	–	50	41

Histoform S

Material	Room temp. approx. +20 °C	Refrigerator +4 °C	Refrigerator on ice 0 °C
Technovit 8100 30:1	69	21	12
Technovit 8100 35:1	67	19	11
Technovit 8100 40:1	65	–	–

Blocking and archiving
Remove the film at room temperature with tweezers once hardening is complete.
The specimens are blocked with Histobloc® and Technovit® 3040 so that they can be removed from the Teflon mould (see page 15). Store blocks that are not needed immediately (for immunohistochemistry) at a cool temperature in plastic bags or similar.

Processing
One obtains the best cutting results with a rotation microtome, with the Technovit Histoblade in combination with the Heraeus knife holder or a hard metal knife (glass diamond knife). Tightly clamp the blocks in the totem cam system on the microtome. Dryly remove the sections with forceps and place in a bath (Aqua dest.). Placed directly on a coated object holder and let dry for 2 hours or more at 37°C. Dry sections that are not needed immediately (for immunohistochemistry) at room temperature at store for a maximum of five days at 4°C.

Object holder coating
For example, submerse the object holder in a solution of 0.5% alcian blue (8GXL Sigma) at 65°C for 15 minutes or liquidly coat the object holder with 0.1% poly-L-lysine (Sigma). All standard coated object holders may be used.

The sections must dry for at least two hours at 37°C. Place the non-deplasticized sections directly in the stain solution or start with enzymatic pre-treatment.

Example

- Enzymatic pre-treatment: Incubate setions for 5-10 minutes in 0.01% trypsin with 0.1% CaCl (calcium chloride) pH 7.8
- Wash multiple times in phosphate buffer (PBS) for five minutes
- Incubate for two hours at 37°C with primary antibody, change multiple times
- Block the endogenous peroxidase with 0.06% hydrogen peroxyde in phosphate buffer (PBS) (30 minutes at room temperature)
- Wash multiple times in phosphate buffer (PBS) for five minutes
- Incubate with the second antibody for 30 minutes at room temperature
- Wash multiple times in phosphate buffer (PBS) for five minutes
- Diaminobenzidine (DAB) as for cyrostat sections
- 10-15 seconds of counterstaining with hematoxylin
- Blue for three minutes under flowing water
- Cover with glycerine gelatine

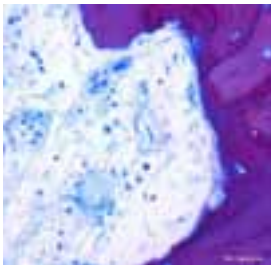
Determination of immuno factors is possible with AP, PAAP, APAAP, ABC, avidin-biotin, streptavidin and immunofluorescence methods.

The use of wetting agent, e. g. Tween, in the rinsing buffer is discouraged. The peroxidase should be dissolved in buffer.

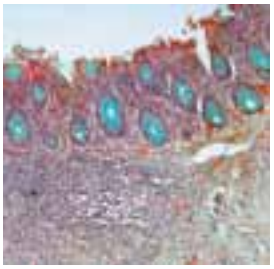
With the constantly changing range of new products for histochemistry and immunohistochemistry, is is always advisable to follow the respective manufacturer instructions.

Section of stent
Elastica Van Gieson

Stent
Elastika Van Gieson



Polynuclear giant cells
Toludin blue staining



Appendix for pentachrome staining in accordance with Movat

Technovit 9100
Sections

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

Technovit 9100 was specifically developed for the embedding of mineralised tissues as well as soft tissue with an expanded study spectrum in light microscopy. The deplasticized sections are suitable for histological overview staining, enzyme chemistry and immunohistological studies, including in-situ hybridisation.

Thin sections for immunohistology can be stuck to glass object holders and deplasticized.

Fields of application

- **Hard-cutting technique for making thin layers**
  Examples: Illiac crest biopsies, smaller, spongy and compact bone tissue specimens

- **Division thin section technique (division procedure in point contact technology)**
  Examples: Tooth/jaw areas with and without implants, non-cemented endoprostheses with shaft bones

- **Combined division-thin section technique and hard-cutting technique (target preparation)**
  Examples: Boundary layer and environment assessment for metal implants and non-cemented endoprostheses

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

Material properties

Polymerisation of the hydrophobic Technovit 9100 occurs by excluding oxygen using a catalyst system made of peroxide and amine. Additional components such as PMMA powder and regulator allow for a controlled polymerisation in the cold (in the range of -2 to -20°C, depending on the volume) that guarantees complete dissipation of the polymerisation heat.

The benefits of the system at a glance

- Polymerisation below freezing
- Reproducibility of the embedding results and reliablity due to constant, documented quality controls
- Uniform block hardening
- The PMMA block remains transparent
- Better results with regard to cutting and staining because Technovit 9100 contains a hydrophilising agent
- Can be used for thin section and the sawing and cutting techniques
- Enzyme histology and immunohistology as well as in-situ hybridisation possible (sections)

Product data

Item No.	Designation	Quantity
64715444	Technovit 9100 Combipack	1 x 1000 ml basic solution 1 x 120 g PMMA powder 8 x 1 g hardener 1 1 x 10 ml hardener 2 1 x 5 ml regulator
66006735	Technovit 9100 basic solution	5000 ml
66010251	Technovit 9100 PMMA powder	1000 g
64709022	Technovit 9100 hardener 1	100 x 1 g
66039185	Technovit 9100 hardener 2	9 x 10 ml
66039184	Technovit 9100 regulator	12 x 5 ml

The components

Technovit 9100 basic solution - component (1)
The Technovit 9100 basic solution is comprised of stablised methyl methacrylate. The hydrophily is improved through the addition of a suitable hydrolising agent. Technovit 9100 basic solution can be used when stabilised and unstabilised.

Technovit 9100 PMMA powder - component (2)
The PMMA powder is used to guarantee a clear decrease in polymerisation shrinkage, a reduction in the polymerisation heat released and a better polymerisation process.

Technovit 9100 hardener 1 - component (3)
Hardening powder 1 is a peroxide compound that starts polymerisation with hardener 2.

Technovit 9100 hardener 2 - component (4)
Hardening liquid 2 acts as a catalyst for hardener 1 to facilitate targeted polymerisation even at very low temperatures [< 0 °C].

Technovit 9100 regulator - component (5)
This is comprised of a reactive organic compound that facilitates a regulated polymerisation with controlled low temperature spikes even for large quantities of polymerisation.

Designation	Quantity	Component No.
Technovit 9100 basic solution stabilised	1 x 1000 ml	1
Technovit 9100 PMMA powder	120 g	2
Technovit 9100 hardener 1	8 bags, each 1 g	3
Technovit 9100 hardener 2	10 ml	4
Technovit 9100 regulator	5 ml	5

Application

Fixation - tissue pre-treatment
Fixation is done for 12 to 24 hours in various fixation solutions depending on the size of the tissue and the antigen/enzyme to be detected. Overfixation must always be avoided.

The following fixation methods are possible for detecting antigens/enzymes:

a) 4% neutral buffered formalin solution (0.1 M phosphate or 0.02 M phosphate buffer for illiac crest biopsies)
b) 10% buffered formalin solution (0.1 M phosphate buffer)
c) Fixation solution in accordance with Schaffer (formol/alcohol)
d) 1.4% paraformaldehyde solution, cold (+4 to +8°C) for 24 - 28 hours (sensitive enzyme detection such as alkaline phosphatase, fixation-sensitive antigens)

Dehydration, intermedium and immersion (pre-infiltration 1-3, infiltration)

Processing may only be done in PE or glass containers!
Dehydration occurs in an ascending alcohol series (dehydration machine) at room temperature. Cavities comprised of white bead polymers that negatively impact cutting and the quality of the section form in insufficiently dehydrated tissue. Xylol is used as an intermedium.

Immersion (pre-infiltration 1-3, infiltration) occurs in 3 phases (in the dehydration machine up to pre-infiltration 2). The specified times and minimum times are based on small, spongy and cortical bone tissue specimens and illiac crest biopsies (the times and volume must be adjusted for larger tissue specimens).

Dehydration, intermedium and pre-infiltration

Phase	Solution	Concen-tration	time/ Temperature
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 basic (stab.) + Hardener 1		> 1 h / RT
Pre-infiltration 3 (Refrigerator)	Technovit 9100 (destab.) + Hardener 1		> 1 h / 4 °C
Infiltration (Refrigerator)	Technovit 9100 (destab.) + Hardener 1 + PMMA powder		> 1 h / 4 °C after 5 days, change solution

TIP:
A standard PMMA granulate can also be used for particularly large specimens (endoprostheses). The amount of required polymerisation solution is thereby reduced.

Destabilisation of the basic solution - processing the components

Technovit 9100 basic solution can be used when stabilised and unstabilised.

The application of destabilised basic solution guarantees that the results for all immunohistochemical studies are analagous to the paraffin histology.

Fill chromatography column with approx. 50 g of Al_2O_3 (active, alkaline, 90) and slowly flow Technovit 9100 basic solution (material number 1) through it. A column filling with Al_2O_3 is able to destabilize 3-4 liters of basic solution. The destabilised solution is portioned into sealable brown glass bottles and stored at +4°C for the ongoing processing (max. 5 days) or kept in storage in aliquots at -15°C to -20°C. Destabilised basic solution can be worked with starting with pre-infiltration 3. When working with destabilised MMA basic solution, a lower amount of peroxide can be used for the infiltration solution and stock solution.

Making the solutions

- Working solution
  Make the pre-infiltration, infiltration and stock solutions according to precise instructions in accordance with the instructions for Technovit 9100. Adhere to the storage temperatures!

- Polymerisation solution
  Cooled stock solutions A and B must be mixed immediately before use in a ratio of 9 parts (v/v) stock solution A (graduated cylinder) and 1 part stock solution B (pipette) in a beaker using a glass stirrer.

Making the working solutions

Component No.	1	2	3	4	5		
Designation	**Basic solution**	**PMMA powder**	**Hardener 1**	**Hardener 2**	**Polymerisation-regulator**	**Processing temperature**	**Storage shelf life**
Pre-infiltration 3	200 ml		1 g			Room temp.	1/2 year at –20 °C
Infiltration	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

Explanation of „ad“: When preparing solutions out of solid substances, the final volume adjustment is made only once all of the substance has dissolved. Please use volumetric flasks.

* When using stablised Technovit 9100 NEW the greater amount of hardener 1 must be used.

Polymerisation
The polymerisation mixture is poured into the pre-cooled embedding form. Place the infiltrated tissue into the form, pour the polymerisation mixture to the brim and subsequently evacuate. Evacuation is done either in the pre-cooled desiccator at 4°C (light 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. Hermetically seal form!
Polymerisation occurs in the range of -2°C to -15°C.

For example:
Embedding form 25 mm (10 ml) and the cradle insert: -2°C to 4°C in approx. 24 hours.

Polymerisation is complete in approximately 24 hours. The polymerisation times depend on the polymerisation volume and the temperature. The greater the volume of the embedding form, the lower the temperature must be! Larger specimens must therefore be hardened at lower temperatures. In the process, adhere to the cold capacity of the explosion-protected cooling device used (freezer in the refrigerator, deep freezer, freezer, freezer well, e.g. for paraffin blocks with lid clip.
Reproducible results for various specimen sizes are achieved in a deep freezer with variable temperatures between -2°C and -25°C with temperature consistency of +/- 0.5°C. Do not open the containers during polymerisation!

Blocking and archiving
Once the specimens have warmed to room temperature after hardening, use Histoform N to block with Histobloc® and Technovit® 3040 (see page 15). First loosen the bolts and remove the lid and film. The block is tightly clamped in the standard object clamp on the rotation microtome for hard-cut sections.
When using round histo-embedding forms the lid and bottom are removed and the specimen is pushed through. It can then be placed directly in the round sample holder on the rotation microtome for hard-cut sections without being blocked.

Processing the polymers
Depending on the question, polymers are processed using the hard-cutting or division thin section technique

- Making hard-cut sections with corresponding hard-cut microtomes
- The same applies to semi-thin sections with the use of glass and diamond knives. The blocks are first trimmed
- Use 16cm hard metal knives with section D
- Use 30% ethanol, so-called cutting fluid, to cut the polymerised Technovit 9100 blocks
- Place sections on coated object holders, stretch with 50% ethanol, so-called cutting fluid, and cover with PVC film
- Soak up excess liquid with filter paper, stack object holders and let dry under pressure (section press) over night at +50°C. Only open press after allowing it to cool. Carefully remove cover film from the cold object holder.

Section deplasticizing

Xylol	2 – 3 x 20 min.	Room temperature
2-methoxyethyl acetate	1 x 20 min.	Room temperature
High-purity acetone	2 x 5 min.	Room temperature
High-purity acetone	2 x 2 min.	Room temperature
Aqua dest.		
Alternative:		
2-methoxyethyl acetate	3 x 20 min.	Room temperature
Descending alcohol series		

3. Division thin section technique
Division with point contact technology and cutting with surface contact or line contact processes with corresponding devices.

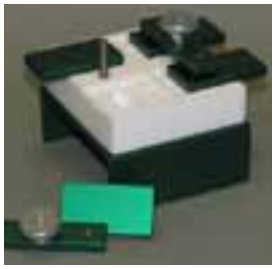
Technical data

Colour	Transparent
Density = spez. weight g/cm³ (DIN 53479)	1,07
Refractive index	
Monomer	1,4175
Polymer	1,4720
Storage temperature	max. 25 °C

Recommended laboratory equipment for the application of the Technovit 9100 system

- Chromatography column
- AL_2O_3 (active, alkaline, 90)
- Adjustable refrigerator
- Glass desiccator
- Vakuum pump
- Magnetic stirrer





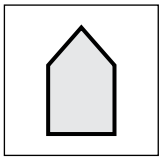

Accessories for Technovit histology technology

Histoforms

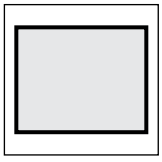
Special embedding forms made of Teflon with a stainless steel bottom for heat dissipation were developed to keep the temperature in the low ranges during polymerisation. Furthermore, the special profile of the embedding forms allows for embedding that is easy to use and cut.

Overview of the benefits:

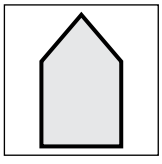
- Embedding material-saving volume
- Easy to cut form (S + N)
- Long life
- Easy to remove the embedding due to the conical form and the Teflon
- Excellent temperature dissipation through the stainless steel bottom
- Precise and easy fastening of the Histobloc support elements due to special opening



Histoform S:
Technovit 7100/8100
10 cavities
Dimensions: B x H x T:
approx. 10 x 16 x 6,5 mm



Histoform Q:
Technovit 7100/8100
for holding large preparations
10 cavities
Dimensions:B x H x T:
approx. 20 x 16 x 10 mm



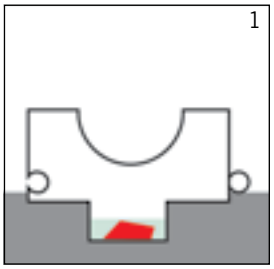
Histoform N:
Technovit 9100
with 4 cover plates
4 cavities
Dimensions: B x H x T:
approx. 12 x 20 x 10 mm

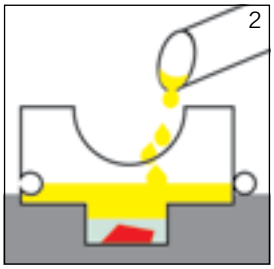
Embedding mould specifically for Technovit 9100

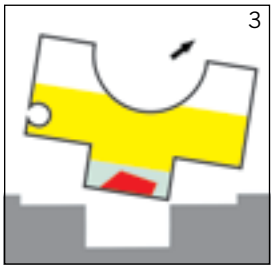
Polyethylene embedding forms 25 mm & „cradle“ insert for the round specimen holder on the rotation microtome. Additional sizes: 15 - 30 - 40 - 50 mm (only cylinder)

Application:

1. Remove bottom of form, stick the cradle and the form together
2. Fill up insert
3. Position specimen in the cradle
4. Fill to brim. Turn on vacuum (approx. 10 min. in the desiccator) max. 200 mbar, turn off vacuum
5. Put lid on (airtight). Put in the refrigerator at -2°C/-4°C







Technovit 3040 / Histobloc

Technovit 3040 is a quickly hardening 2-component plastic with a methyl meth acrylate base (MMA). It is used to block or fasten embedded plastic specimens with the Histobloc or for socket embedding.
The chemical composition allows for a stable, permanent bond with the hardened block Technovit 7100, Technovit 8100 or Technovit 9100.

Histobloc – support elements
The Histobloc supports are adjustable to Histoforms S + Q or for Histoform N.

Blocking
Once Technovit 7100, Technovit 8100 or Technovit 9100 has hardened, the Histobloc support element is placed in the opening in the Teflon embedding form Histoform S/Q or Histoform N intended for this purpose (Image 1).
If Technovit 8100 was used for embedding, the cover film must be removed from the specimen before the Histobloc support element is put into place.
For Histoform N, the aluminium lid is removed and the PE film is removed.
Mix Technovit® 3040 until it is as viscous as possible (mixing ratio 2-3 volumetric parts of powder to 1 volumetric share of liquid). Pour the mixed material into the opening in the Histobloc®. (Image 2)

Technovit hardens and the Histobloc tightly bonds to the specimen in 5 - 10 minutes, depending on the room temperature.
The specimen can now be removed from the form and is ready to be cut (Image 3). Through blocking with the very hard Technovit® 3040, the elastic Technovit 7100 or Technovit 8100 is stabilised in such a way that even the thinnest sections are possible.

Clamp the specimen on the microtome.
The square form of the Histobloc allows for the specimen to be directly clamped in the rotation microtome‘s totem cam system.

Note for Technovit 9100:
When using the round Histo embedding form for Technovit 9100, the specimen can be clamped directly in the round specimen holder; blocking is not necessary.

Storing and archiving specimens 7100 and 8100
The Histoblocs can be stacked on top of each other for better archiving. Thus, serial numbering and space-saving storage is possible.

For histochemical and immunohistochemical studies it is recommended to store the blocks in PE bags at +4°C or longer at -20°C




Technovit Histoblade and blade holder

The Technovit Histoblade is specially suited for cutting tissue samples embedded in HEMA (2-hydroyethyl methacrylate) and materials with industrial applications with a section thickness of up to 1µ.
Thus, coupled with the exceptionally stabile blade holder, a cheap alternative to other knives is available.

The benefits:
- Heavy, stabile design
- Easy to use
- Good cutting results for up to 1µ section thickness
- Optimised section quality
- Improved durability

The knife angle when using the Technovit Histoblade should be 9° for microm/thermal rotation microtomes. You need an „NR“, „SL“ or „N“ blade holder. For Reichert-Jung/Leica-Rotation microtomes at 0°. You need an „NZ“ blade holder. Please contact your microtome provider if you have any questions.

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

Object holder press

Press made out of metal for flat pressing of the MMA sections on the object holder during the drying process. At least 50 - 70 object holders fit in the object holder press (place filter paper between each object holder).

Cover films

PE film for covering MMA sections on the object holder after stretching (e.g. Technovit 9100), format: 25 x 75 mm
The film is also used to cleanly seal Histoform N with the aluminium plate.

Manual specimen holder and inserts

The manual specimen holder is an important aid for making plano-parallel thin sections and the target preparation for polished thin sections.

Manual specimen holder H
Manual specimen holder „H“ (= histology) is used to make thin sections.
Press either a round or a square glass or PMMA object holder on which the specimen (plano-parellel) can be placed with transparent adhesive onto the flat stainless steel surface of the manual specimen holder.
A drop of water, oil or Canada balsam may be used. Specimens can also be stuck directly on the metal surface.
The nonius setting on the manual specimen holder‘s adjusting ring makes it possible to achieve defined material removal. The locking ring prevents the specified dimensions from being altered during the cutting process. The ceramic ring guarantees safe cutting up to the desired level and prevents overcutting.

Manual specimen holder M plus inserts
A polished thin section can be made for the target preparation from an embedded specimen. For this, one uses manual specimen holder „M“ (= materialography). The standard diameter is 40 mm. The 32 mm, 30 mm and 25 mm inserts can be used in the standard holder for smaller diameters.

If a thin section is made out of a polished thin section, it is possible to put insert H in the manual specimen holder M (standard 40 mm). Then proceed as stated in the „Manual specimen holder H“ section.

Delivery units

Item No.	Description/content
66038248	Histoform N, embedding form made of Teflon, cover plates made of, 1 unit
64708992	Histoform Q, embedding form made of Teflon, 1 unit.
64708991	Histoform S, embedding form made of Teflon, 1 unit.
64708955	Embedding form 25 mm, 1 pk. (3 cylinders, 3 covers)
66009903	Cradle insert for embedding form 25 mm, 1 pk. (3 units)
64713126	Embedding mould 15 mm, 1 pk. (3 cylinders, 3 covers)
64708956	Embedding mould 30 mm, 1 pk. (3 cylinders, 3 covers)
64708957	Embedding mould 40 mm, 1 pk. (3 cylinders, 3 covers)
64713127	Embedding mould 50 mm, 1 pk. (3 cylinders, 3 covers)
64708805	Technovit 3040 yellow Combipack, 1 x 100g / 1 x 80 ml
64708806	Technovit 3040 yellow powder, 1000g
66022678	Technovit universal liquid, 500 ml
64712817	Histobloc for Histoform N, 50 units
64708995	Histobloc for Histoform S & Q, 100 units
66021102	Mixing bowl
64701106	Spatula
64701107	Scoop
64708996	Microtome blade holder, 17 cm
66045730	Technovit Histoblade 1 x 50 units
66031158	Manual specimen holder H
66031155	Manual specimen holder M Ø 40 mm
66031159/60/61	Insert for manual specimen holder M 25, 30, 32
66031157	Insert H
64712819	Object holder press
64712818	Polyethyethylen films 75 x 25 mm, 200 units

Basic information for using Technovit Histotechnology

Usage instructions
The described embedding systems deal with chemicals; thus, the relevant laboratory regulations and guidelines that regulate the handling of chemicals must be adhered to.

- Do not bring monomers into contact with metallic equipment (e. g. metallic stirrer or tweezers)
- Clean containers without detergent
- Clean MMA contaminated containers with acetone/alcohol
- Polymerise remains in solvent waste container or plastic waste container
- Subsequently use PE or glas containers
- Clean chromatography column (for destabilising Technovit 9100 basic solution) directly after use with acetone/alcohol
- Disposing of aluminium oxide with stabilizer: evaporate on aluminum foil under the flue, put in residual waste or dispose of it in a waste container for contaminated substances
- Use a magnetic stirrer without hotplate to dissolve the PMMA powder in Technovit 9100

The corresponding safety data sheets can be retrieved at: www.technovit.de/Service/Downloads

Technovit 7100
Routine staining, enzyme histochemistry according to Gerrits

HEMATOXYLIN-EOSIN

Staining process

1. Stain the sections in hematoxylin in accordance with Gill* (filtrate the dye solution)	15 min.
2. Blue in tap water	10 min.
3. Rinse in aqua dest.	
4. Counterstain sections with Eosin	2–5 min.
5. Dehydrate through ethanol 96% and 100%	
6. Clarify with xylen and cover in Eukitt	

Result

Nucleus	blue
Basophilic cytoplasm	blue
Acidophilic cytoplasm	pink
Muscle tissue	pink
Connective tissue	pink

Solutions

Hematoxylin in accordance with Gill

Hematoxylin (C.I. 75290)	6 g
Sodium iodate	0,6 g
Aluminum sulphate	52,8 g
Aqua dest.	690 ml
Ethylene glycol	250 ml
Glacial acetic acid	60 ml

Eosin

Eosin Y-(alcoholic) C.I. 45380	0,5 g
Ethanol 96 %	100 ml
Glacial acetic acid	2 drops

*After staining with hematoxylin (1) the plastic matric can be decolorised with 0.5 ml of HCL (36%) in ethanol 70%: briefly submerse and then quickly process in tap water (2).

PERIOD ACID SCHIFT (PAS)

Staining process

1. 0,4 % periodic acid	30 min., 56 °C
2. Rinse in tap water	
3. Aqua dest. rinse 3x	
4. Schiff's reagent	15 min.
5. Rinse thoroughly in tap water	
6. Rinse in aqua dest.	
7. Counterstain sections with hematoxylin in accordance with Gill	10 min.
8. Blue in tap water	10 min.
9. Dehydrate, clarify with xylen and cover in Eukitt	

Note: To avoid a specific pink sheen, one can rinse with sulphite water instead of tap water (5), see also Feulgen.

Result:

Nucleus	blue
Glycogen	violet / red
Basement membranes	violet / red
Mucin	violet / red

Solutions:

Schiff's reagent

Solution 1: Pararosaniline (C.I. 42500)	0,5 g
1 N hydrochloric acid	15 ml
Solution 2: Potassium metabisulphite ($K_2S_2O_5$)	0,5 g
Aqua dest.	85 ml

Mix solution 2, solution 1. After 24 hours (in the dark) the light brown solution is decolorised with 200 mg of bone black (approx. 2 min.) and subsequently filtrated.
Store the colorless reagent (leucofuchsin) in the refrigerator.
Gill's hematoxylin: see Hematoxylin-Eosin

FEULGEN

Staining process

1. Hydrolise in hydrochloric acid 5 N	20 min. ZT
2. Rinse in aqua dest. 3 x	
3. Schiff's reagent	15 min.
4. Sodium hydrogen sulphite 0.5%	3 x 2 min.
5. Rinse thoroughly with tap water	
6. Dehydrate, clarify with xylen and cover in Eukitt	

Result

DNS	violet / red
Other tissue elements	colourless

Solutions

Schiff's reagent

Hydrochloric acid 5 N

Fill up with 42 ml of hydrochloric acid 36% up to 100 mlNatriumhydrogensulfit

$NaHSO_3$	0,5 g
Aqua dest.	100 ml

GIEMSA

Staining process

1. Stain sections in the Giemsa solution (20 %) (Giemsa Merck: Dilute 1:5 with aqua dest.)	1,5 hrs. ZT

2. Briefly in acetic acid solution:
   4 drops to 100 ml of aqua dest. 2 seconds
3. Submerse in alcohol 96%
4. Submerse in alcohol 96%
5. Isopropanol 3 x 2 min.
6. Clarify with xylen and cover in Malinol

Results

Nucleus	violet
Cytoplasm	blue
Erythrocytes	pink

PRUSSIAN BLUE REACTION IN ACCORDANCE WITH PERLS

Staining process

1. Potassium ferrocyanide 15 min.
   First warm up the solution to 60°C
   and then filtrateanschließend filtrieren
2. Rinse in aqua dest.
3. Safranin O. 0,2 % 2–5 min.
4. Rinse in acetic acid 1 %
5. Dehydrate, clarify with xylen and cover in Eukitt

Results

Nucleus	red
Hemosiderin	blue / green

Solutions

Potassium ferrocyanide solution

Potassium ferrocyanide	1 g
Aqua dest.	50 ml
Hydrochloric acid 2 %	50 ml

Safranin-Solution

Safranin O. (C.I. 50240)	0,2 g
Acetic acid 1 %	100 ml

PERIODIC ACID METHENAMINE SILVERC (PAMS) ACCORDING TO JONES

Note: It is recommended to stick on the plastic sections with Mayer‘s albumin.

Staining process

1. Periodic acid 1 % 30 min.
2. Rinse in aqua dest. 3x
3. Methenamine silver solution 60 min., 60 °C
4. Rinse in aqua dest., microscopic test.
   Sections that have been too weakly
   stained again in 3
5. If the sections refuse to dissolve
   despite pre-treatment, dry them on
   a plate at 60°C in accordance with Point 4.
6. Gold chloride 0,2 % 1–2 min.
7. Rinse in aqua dest.
8. Sodium thiosulphate 2 % 5 min.
9. Rinse in tap water
10. 10. If necessary, counterstain
    with HE or safranine O
11. 1Dehydrate, clarify with xylen and cover in Eukitt

Result

Basement membranes	brown / black

Solutions

Methenamine silver tock solution

a) Hexamethylenetetramine 3 %	100 ml
b) Silver nitrate 5 %	5 ml

a) and b) can be stored separately.

Methenamine silver stain solution

Stock solution	50 ml
Borax 5 %	5 ml

Periodic acid: 1 % (Sigma No. P 7875)

Gold chloride: 0,2 %

Sodium thiosulphate solution: 2 % ($Na_2S_2O_3.5H_2O$)

DETERMINING ENZYME ACTIVITY

Determination of the enzyme activity in tissues that are embedded in 2 hydroxyethyl methacrylate (GMA) - in particular Technovit 7100

Freshly removed tissue is fixated in 4% neutral formaldehyde at 4°C for two hours (immersion). If perfusion fixations are made, very brief fixation times can be adhered to and the enzyme activity is better maintained.

Rinsing fluid

0.1 M cacodylate buffer pH 7.4; the material can possibly be left overnight at 4°C.

Dehydration

1. Alcohol 70 % acetone 70 %, 30 min. at 4 °C
2. Alcohol 96 % acetone 96 %, 30 min. at 4 °C
3. Alcohol 100 % acetone 100 %, 30 min. at 4 °C

Pre-infiltration

4. Alcohol 100 % Technovit 7100 1:1, 2 hrs. at 4 °C
   or
   Acetone 100 % Technovit 7100 1:1, 2 hrs. at 4 °C

Infiltration
Technovit 7100, 12 hrs. at 4 °C

Polymerisation
15 parts Technovit 7100 (solution A)
1 part Technovit 7100 hardener II, at 4 °C

The tissue can be embedded in Histoforms S or Q, or in the Sorvall embedding system. Because polymerisation starts at 4°C, it will occur slower than at room temperature. A polymerisation time of 12 hours at 4°C must be adhered to ensure polymerisation.
the 2-µ sections are also dried at room temperature on aqua dest. Enzyme actions can be made without removing the plastic matrix.

Note: It is difficult to detect dehydrogenases.

ALKALINE PHOSPHATASE IN ACCORDANCE WITH BURSTONE

Staining process

1. Incubate the plastic sections in the incubation medium Note: In many cases a 2-hour incubation period is sufficient.	1–3 hrs.
2. Rinse in aqua dest.	2 min.
3. Counterstain the sections with nuclear fast red	5–10 min.
4. Rinse in aqua dest.	
5. Air dry	
6. Cover in malinol	

Result

Nucleus	red
Enzyme activity area	blue

Note: In this reaction the choice of medium used to cover the material is significant because crystals formation may occur in the reaction product.

Solutions

Buffer solution

0.2 M tris-(hydroxymethyl)-aminomethane	2,4 g
Aqua dest.	100 ml

Set the pH value to 8.9 with diluted HCL and store the buffer at 4°C.

Incubation medium

Naphtol AS-MX phosphate, disodium salt (Sigma)	5 g
N,N dimethylformamide	0,25 ml
After dissolving, add:	
Aqua dest.	25 ml
Buffer solution (pH 8,9)	25 ml
$MgSO_4.7H_2O$ 10 %	2 drops
Fast Blue BB (Sigma)	30 mg

Shake well and then filtrate before using.
Note: Always freshly prepare the incubation medium.

ATP-ASE (WACHSTEIN AND MEISEL)

Staining process

1. Incubate the plastic sections in the incubation medium (filtrate before using) Note: In many cases a 2-hour incubation period is sufficient.	1–3 hrs., 37 °C
2. Rinse in aqua dest.	2 min.
3. Sodium sulphide solution	30 sec.
4. Rinse in aqua dest.	
5. Counterstain the sections with nuclear fast red	5–10 min.
6. Rinse in aqua dest.	
7. Air dry	
8. Cover with Eukitt or malinol	

Result

Nucleus	red
Enzyme activity area	brown

Solutions

1. Tris maleic acid buffer pH 7.2 solution A

Maleic acid	29 g
Tris-(hydroxymethyl)-aminomethane	30,3 g
Aqua dest.	500 ml

Add 2 g of activated carbon, shake for ten minutes and filtrate. Then add 40 ml of the stock solution A, 20 ml 1N NaOH, and fill with aqua dest. up to 100 ml (pH 7.2).

2. Lead nitrate solution

Lead nitrate	2 g
Aqua dest.	100 ml

3. Magnesium sulphate solution

$MgSO_4.7H_2O$	1,2 g
Aqua dest.	100 ml

Incubation medium

Aqua dest.	22 ml
Disodium adenosine-5-triphosphate (Boehringer, Mannheim)	25 mg
Tris maleic acid buffer pH 7,2	20 ml
Magnesium sulphate solution	5 ml
Lead nitrate solution (add by drops), heat to 42°C and filtrate)	3 ml

Sulphide solution

Sodium sulphide	2 g
Aqua dest.	100 ml

Adjust the pH value to 7.0-7.5 with 1 N of HCL (verify with pH paper)

Technovit 9100
Routine staining, immune reactions, enzyme histochemistry, in-situ hybridisation

The following staining and detection reactions are only important examples of processing hard-cut sections. They also apply to MMA thin sections.
Reagents, antibodies, probes, detection seystems are variable.

1. Routine staining

Counterstaining the sections for immunohistochemistry and enzyme histochemistry

- Hematoxylin n. Mayer flowing — 30 sec./RT
  water in tap water transfer to — 10 min./RT
  aqua dest.
- Rinse nuclear fast red in aqua dest. — 10 min./RT
- Methyl green (cleaned; see Romeis) — 10–20 min./RT
  Rinse with aqua dest.

HE staining

- Same as staining paraffin sections

Giemsa staining

- Deacrylate sections
- Giemsa sol. (Mix fresh!) — 30–40 min./RT

Differentiate and dehydrate
- Acetone / xylol (95 : 5)
- Acetone / xylol (70 : 30)
- Acetone / xylol (30 : 70)
- Xylol

Masson Goldner staining

- Deacrylate sections
- Haemalaun (Mayer) — 10 min./RT
- Tap water
- Ponceau acid magenta azophloxin — 45 min./RT
- 1% acetic acid
- phosphomolybdic acid / Orange G — 7 min./RT
- 1% acetic acid
- Light green — 40 min./RT
- 1% acetic acid
- Ascending alcohol series
- Xylol
- Cover with Eukitt or similar

2. Complete immune reaction

Antibody incubation

- Reinse in 0.01M phosphate buffer (pH 7.4)
- Primary antibody — 16 h/4 °C or
  Diluted in DAKO Antibody Dilutent — 30–45 min./RT
- Rinsing buffer
- DDAKO EnVision Polymer
  (GAM/GAR), AP paired — 30 min./RT

Detection reaction

- Rinsing buffer
- Substrate chromogen solution:
  Fast Red — 15–20 min./RT
- Counterstaining with hematoxylin to Mayer

3. Execution of enzyme histochemistry

Alkaline and acid phosphatase

- Rinse in 0-1M tris buffer (pH 9.4) — 10 min./RT
- Incubation in the reaction solution — 2 h/37 °C
  0.1MTris buffer (pH 9.4)
  Real blue salt
  Naphthol-AS-BI-phosphate
- Rinse in aqua dest.
- Rinse in 0-1M acetate buffer (pH 5.6) — 10 min./RT
- Incubation in the reaction solution — 1 h/37 °C
  0.1M acetate buffer (pH 5.6)
  Hexanium-pararosaniline solution
  Naphthol-AS-BI-phosphate
- Rinse in aqua dest.
- Refixate in 4% formalin — 2–3 h/RT
- Rinse in tap water
- Counterstain with methyl green

Naphthol-AS-D chloroacetate esterase (ASD)

- Reinse in 0.01M phosphate — 5 min./RT
  buffer (pH 7,4)
- Incubation in the reaction solution — 1 h/RT
  0.01M phosphate buffer (pH 6.5)
  Naphthol-AS-D-Chloroacetate
  Hexanium-pararosaniline solution
- Rinse in aqua dest.
- Counterstain with hematoxylin to Mayer

*RT = Room temperature

Technovit 9100
Short Instructions
In-situ hybridisation for sections

1. Prepare approx. 5 µm-thick sections
2. Section deplastization at room temperature
   - Xylol 2 x 20 min.
   - 2-methoxyethyl acetate (2-MEA) 1 x 20 min.
   - High-purity acetone 2 x 5 min.
   - Aqua dest. 2 x 5 min.

   If necesary deplasticize for more time, also without 2.MEA
3. Edge sections
4. Block the endogenous peroxidase 3% H_2O_2 in methanol 30 min.
5. Aqua dest. 2 x 5 min.
6. Enzymatic digestion
   - Fast enzyme 10 min. bei RT.
   - Pronase 0,1%, 37 °C 10 min.
7. Aqua dest. minimum 10 min.
8. Fresh aqua dest. Possibly also over night
9. Let sections dry
10. Apply probe to the sections, cover with cover glass and seal with Fixogum
11. Place sections in the hybridizer and hybridise for 2 hours at 55°C
12. Remove sections from device, remove Fixogum
13. Place sections in wash buffer 2 x 2 min. at RT

Detection:

1. Place AP anti biotin on the sections 30 min., 37 °C
2. Wash in buffer 2 x 2 min., RT
3. Place AP substrate on the sections 30 min., 37 °C
4. Wash in buffer 2 x 2 min., RT
5. Place HRP anti dig. on the sections 30 min., 37 °C
6. Wash in buffer 2 x 2 min., RT
7. Place HRP substrate on the sections 30 min., 37 °C

   HRP substrate from kit for formalin and plastic-fixated illiac crests:

   HRP substrate for Shäfer fixated illiac crests:
   - 10,5 mg of 3-Aminoethylcarbazole (Sigma a 5754)
   - 1 ml DMSO
   - in 50 ml acetate buffer pH 5,6 (0,1 molar)
   - 5 µl H_2O_2
8. Rinse sections under running water and if necessary counterstain briefly with diluted hemalaun.
9. Cover with water

NOTE:
Ask the corresponding probe manufacturer for additional instructions oh ISH.

Source: Self-experiment with reagents completed by Zytomed Systems GmbH

Reagents

Buffer

2M SODIUM ACETATE STOCK SOLUTION

74,13 g of sodium acetate
5,5 ml of glacial acetic acid
ad. 500 ml Aqua dest.

0,1M SODIUM ACETATE BUFFER (pH 5,6)
50 ml of 2M sodium acetate stock solution
ad. 1000 ml Aqua dest. (adjust pH to 5,6)

1M PHOSPHATE STOCK SOLUTION
112,5 g Na_2HPO_4
30 g KH_2PO_4
ad. 1000 ml Aqua dest.

0,1M PHOSPHATE BUFFER (pH 6,5)
100 ml of 1M phosphate stock solution
ad. 1000 ml Aqua dest. (adjust pH to 6,5)

0,01M PHOSPHATPUFFER (pH 7,4)
10 ml of 1M phosphate stock solution
ad. 1000 ml Aqua dest. (adjust pH to 7,4)

0,04M PHOSPHATE BUFFER + 10% SUCROSE (pH 7,4)
40 ml of 1M phosphate stock solution
100 g of sucrose
10 ml of 10% NaN_3 solution
ad. 1000 ml Aqua dest. (pH 7,4)

1M TRIS STOCK SOLUTION
121,14 g of tris
ad. 1000 ml Aqua dest.

0,1M TRIS BUFFER (pH 9,4)
100 ml of 1M tris stock solution
ad. 1000 ml Aqua dest. (adjust pH to 9,4)

Fixation solutions

Buffered 4% FORMALIN SOLUTION
100 ml of 37% formol
4 g of NaH_2PO_4. H_2O
6,5 g of Na_2HPO_4
ad. 1000 ml Aqua dest. (pH 7,0)

8% PARAFORMALDEHYDE STOCK SOLUTION
40 g of paraformaldehyde
ad. 500 ml Aqua dest.

1.4% PARAFORMALDEHYDE SOLUTION
35 ml of 8% paraformaldehyde stock solution
65 ml of Aqua dest.
100 ml of 0.04M phosphate buffer +10% sucrose
(pH 7,4)

Reaction batches

FAST RED SOLUTION
Put 3 ml of substrate buffer in a plastic tube
Put 1 Fast Red tablet in the solution and dissolve
Add 120 µl of levamisol and mix
Sol. has a shelf life of 1 hour

REACTION SOLUTION: ALKALINE PHOSPHATASE
50 ml 0,1M tris buffer (pH 9,4)
50 mg of Real Blue salt
25 mg of naphthol-AS-BI phosphate (dissolved in
0,5 ml of DMSO/Triton X 100)

REACTION SOLUTION: ACID PHOSPHATASE
50 ml of 0.1M sodium acetate buffer (pH 5,6)
500 µl of hexonium pararosaniline (250 µl 4 %
pararsonanilin in 2N HCl + 250 µl of 4 %
sodium nitrate in aqua dest.; vortex for 1 min.
let react for 5 min.)
25 mg of naphthol-AS-BI-phosphate (dissolved in
0.5 ml of DMSO/Triton X 100)

REACTION SOLUTION: ASD-CHLOROACETATE ESTERASE
50 ml of 0.1M phosphate buffer (pH 6,5)
15 mg of naphthol AS-D chloroacetate (dissolved in
of DMSO/TritonX 100)
250 µl of hexonium pararosaniline

Staining solutions

GIEMSA SOLUTION
3% sol., make using the stock solution (Merck)
1-2 drops of 1% acetic acid

LIGHT GREEN
1 g light green yellowish
2 ml glacial acetic acid
ad 1000 ml Aqua dest.

PHOSPHOMOLYBDIC ACID / ORANGE G
30 g Phosphomolybdic acid
ad. 500 ml Aqua dest.
20 g Orange-G
ad. 500 ml Aqua dest.
– Mix both solutions
– Filtrate

PONCEAU ACID MAGENTA AZOPHLOXIN
100 ml Masson sol.
20 ml Azophloxinlsg.
880 ml 0.2% acetic acid

Masson sol.: 1 part sol. A + 2 parts sol. B

Sol. A: 1 g of acid magenta (magenta-S)
ad. 100 ml Aqua dest.
– boil
1 ml of glacial acetic acid
– Filtrate

Sol. B: 2 g of Ponceau de Xylidine
ad. 200 ml Aqua dest.
– boil
2 ml of glacial acetic acid
– Filtrate

AZOPHLOXIN SOLUTION
0,5 g azophloxin
ad. 100 ml Aqua dest.
2 ml glacial acetic acid

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