

Technovit® 9100 NEW

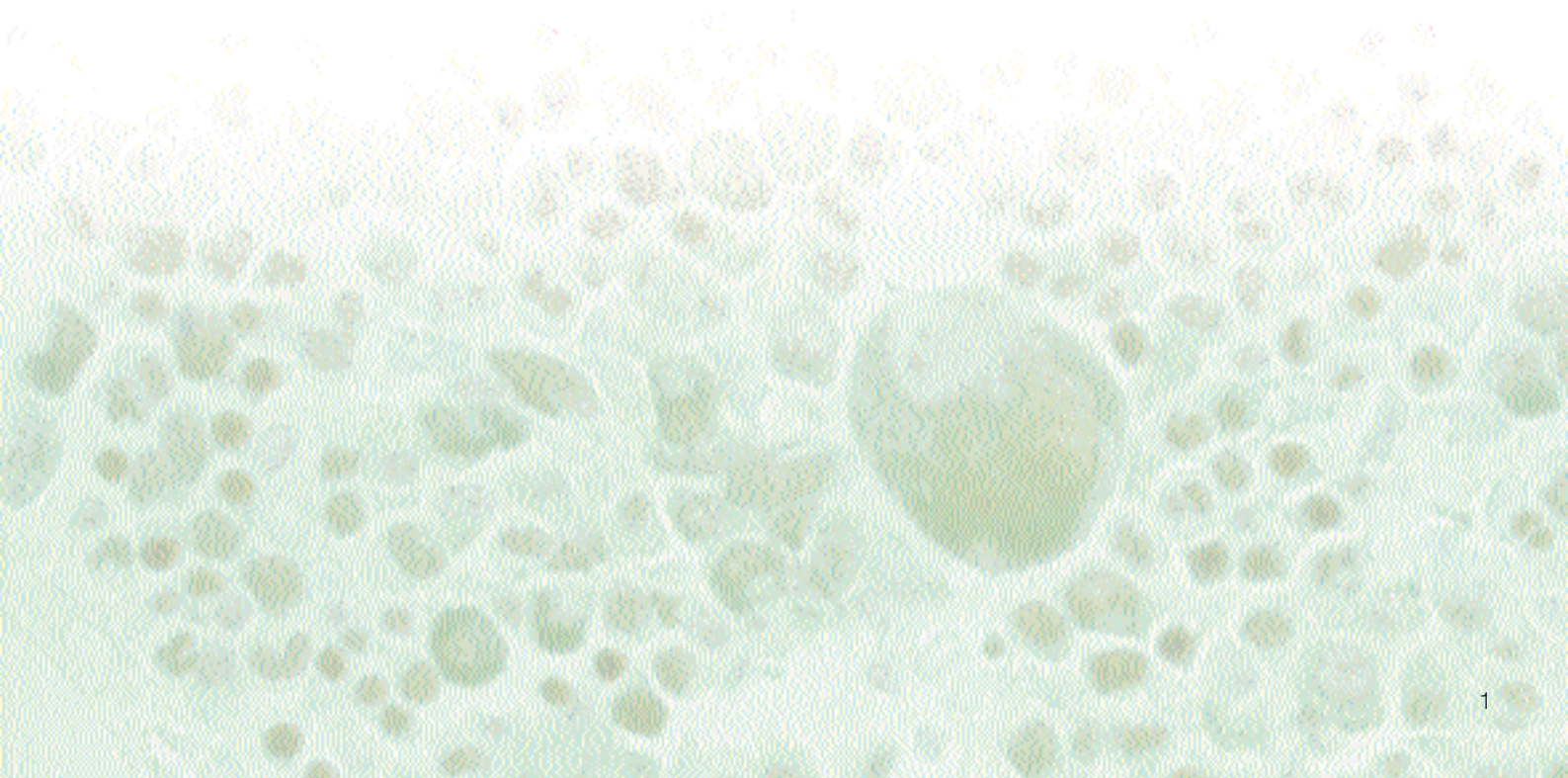
Polymerisation system for embedding mineralised and soft tissues for use in classical histology as well as immuno- and enzyme-labelled histochemical techniques, including in-situ hybridisation.





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1. Areas of Application

Technovit® 9100 NEW is a polymerisation system based on methyl methacrylate (MMA) which hardens at low temperature. It has been specially developed for embedding mineralised tissues with extensive possibilities of staining for light microscopy.

1.1 Heavy duty microtome technique for preparing thin sections

– for example pelvic biopsies, small low- and high-density bone samples.

1.2 Cutting-grinding and micro-grinding system (contact point technique)

– for example jaw and teeth segments with and without implant.

1.3 Combined contact point and heavy duty microtome techniques (Target Preparation)

– for example interface regions and examination of tissues surrounding metal implants and cement-free endoprostheses (target tissue preparation)

Properties

The chemical polymerisation of Technovit 9100 NEW takes place under the exclusion of oxygen with the aid of a catalyst composed of a peroxide and an amine component. Additional components such as PMMA-powder and a regulator allow for a controlled polymerisation by temperatures between -2 and -20 °C, which guarantee a dispersion of the heat generated during polymerisation. The polymerisation time is between 18 and 24 hours using a volume between 3 and 15 ml for the above temperature range. After necessary pretreatment of the tissue – all current staining methods can be applied to the MMA-sections after removal of the polymer. These include classical staining as well as immuno- and enzyme-histochemistry. In-situ hybridisation (ISH) techniques can also be performed.

The polymerisation system Technovit 7200 VLC – developed for classical cutting-grinding and micro-grinding systems (contact-line technique) – retains its proven range of applications and is especially recommended for use with cemented endoprostheses and implants.

Technovit 7100 and Technovit 8100 which are based on glycol-methacrylate are especially suitable for small organ biopsies with thin- and semi-thin section techniques and have a well-known wide spectrum of applications.

The embedding systems complement each other with respect to the diagnostic and scientific questions to be answered.

2. System Components of Technovit 9100 NEW

Component	Amount	Component Number
Basic solution (stabilised)	1000 ml	1
PMMA-Powder	120 g	2
Hardener 1	8 Sachets á 1 g	3
Hardener 2	10 ml	4
Polymerisation regulator	5 ml	5
PMMA-Granulate, EXAKT*	500 g	6

*EXAKT – Trade Mark of EXAKT-Apparatebau GmbH & Co. KG, D-22851 Norderstedt

2.1 Basic Solution.

The basic solution Technovit 9100 NEW is composed of monomers – organic molecules with at least one carbon-carbon double bond.

The stabiliser contained in the kit is responsible for the storage-stability. Hydrophilic properties are improved by addition of a special hydrophilic-generating agent.

Technovit 9100 NEW can be used either in the stabilised or destabilised form (see below under chapter 3).

2.2 PMMA-Powder.

The powder is an internal filler and is made up of PMMA-micropellets. It is used

- to reduce the polymerisation-shrinking effect markedly
- to reduce the heat generated during the polymerisation process as well as
- to improve the quality of the polymerised product.

2.3 Hardener 1

Hardener powder 1 is one of the components of the polymerisation initiation system. It is a derivative of dibenzoyl peroxide which – in combination with Hardener 2 – starts the polymerisation reaction.

2.4 Hardener 2

This liquid hardener is the second component of the initiation system. It works catalytically upon hardner 1 to allow a controlled polymerisation even at temperatures below 0 °C.

2.5 Regulator.

The regulator is composed of a reactive organic compound which allows a controlled polymerisation – even with large volumes of polymer – without large increases in the temperature during the polymerisation reaction.

2.6 PMMA-Granulate, EXAKT

This granulate acts as an additional internal filler when larger amounts (500-1000 ml) of polymer are to be used – as for example in the case of a femur shaft with non-cemented endoprostheses. The amount of monomer (basic solution) is thereby reduced, at the same time making the polymerisation easier to control.



Technovit 9100 NEW System Components



PMMA-Granulate



Chromatography column

3. Using the Components

The basis solution Technovit 9100 NEW can be used in stabilised or destabilised form. The use of destabilised Technovit 9100 NEW basis solution guarantees results for all immunohistochemical methods analogous to those using paraffin sections.

3.1 Destabilising the Basic Solution

Fill a chromatography column with 50 g aluminium oxide and allow the Technovit 9100 NEW basis solution (Component No. 1) to flow slowly through it. A column prepared as above is sufficient to destabilise 3 – 4 litres of basis solution. Store the destabilised basis solution in portions in stoppered brown glass bottles and store either at 4 °C for shorter periods or at -15 °C to -20 °C for longer periods of time.

3.2 Preparation of a ready-to-use solution from the Components 1-5 of the Technovit 9100 NEW Kit.

Procedure: To prepare the solutions for preinfiltration, infiltration and the stocksolutions, see the descriptions in the user instructions. Please note the storage temperatures!

Component number	1	2	3	4	5	
Component	Basic solution	PMMA Powder	Hardener 1	Hardener 2	Regulator	Storage
Pre infiltration	200 ml		1 g			Room temperature
Infiltration	ad 250 ml	20 g	1 g / 2 g*			4 °C
Stock solution A	ad 500 ml	80 g	3 g / 4 g*			4 °C
Stock solution B	ad 50 ml			4 ml	2 ml	4 °C

*When using stabilised Technovit 9100 NEW the increased amounts of Hardener 1 must be used

3.3 Preparation of the Polymerisation Mixture.

Cooled stock solutions A and B should be mixed in the proportion 9 parts A (measuring cylinder) plus 1 part B (pipette) directly before use. This should be performed in a beaker using a glass rod to stir the mixture. The samples are then positioned in containers and completely covered with polymerisation mixture, placed in a cooled dessicator and a partial vacuum produced – for example using a water pump – and left to stand at 4 °C for ca. 10 minutes. The resulting blocks are then placed in a sealed container and left to polymerise at between –8 °C and –20 °C.

3.4 Polymerisation.

The polymerisation process takes place at –8 °C to –20 °C . The samples are then allowed to stand at 4 – 8 °C (Refrigerator) for at least one hour before being allowed slowly to come to room temperature.



Refrigerator with temperature-circuitry

The polymerisation times are dependent on the volumes of polymerisation mixture used and of the constancy of the temperature at which polymerisation is carried out. Larger samples should be polymerised at lower temperatures. The cooling capacity of the refrigerator (ice box, deep freezer, freezer-unit with lid) and the volume of polymerisation solution should be taken into account - for example with paraffin blocks with lids – when preparing for polymerisation. Reproducible results are achieved in the refrigerator by temperatures from ± 0,5 °C when EBS-Temperature-Regulator is used.

gelatine capsules 0.2 ml	between -8 °C and -15 °C	18 to 24 h
teflon mould 3 ml capacity	between -8 °C and -15 °C	18 to 24 h
PVC capsule 15 ml capacity	between -8 °C and -15 °C	18 to 24 h
larger PVC moulds (200 – 300 ml)	between -15 °C and -20 °C	24 to 48 h
very large PVC moulds 500 – 1000 ml)	between -20 °C and -25 °C	48 h

When the prepared samples have been brought to ambient temperature after polymerisation is complete, they can be mounted on blocks using Technovit 3040 in order to remove the samples from the teflon moulds.



Polymerization containers and specimen holder

4. Preparation of tissues before embedding

4.1. Fixing the tissues

The time for fixing is usually between 12 and 24 h and takes place in different solutions depending not only upon the composition of the specimen but also on the antigen or enzyme to be labelled. The following methods of fixation can be used when detecting antigens or enzymes.

- 4% neutral buffered formalin (0.1 mol/l phosphate buffer – or 0.02 mol/l phosphate buffer for pelvic biopsies)
- 10% buffered formalin (0.1 mol/l phosphate buffer)
- Fixation according to Schaffer (Formol/Alcohol).
- 1.4% paraldehyde solution at 4 – 8 °C for 24 – 48 h. (suitable for sensitive detection of enzymes such as alkaline phosphatase and for antigens sensitive to denaturation / structural changes.)

4.2 Dehydration, Intermediate and Immersion (Preinfiltration steps 1-3, Infiltration).

Dehydration is performed in increasing concentrations of alcohol and can be performed automatically in a suitable device at ambient temperature. If dehydration is incomplete, so-called "Lunker-Stellen" containing pearls of white polymer develop, which can negatively influence the cutting of the block as well as the quality of the sections obtained. Xylol is used as intermediate solution.

Immersion (Pre-infiltration steps 1-3 plus infiltration) takes place in three stages. Pre-infiltration steps 1 and 2 can be performed automatically in a suitable dehydration device. The times given in the table below are for small spongy and cortical bone samples and pelvic biopsies. For large tissue samples, the times and volumes should be increased proportionally.

Step	Solution	Concentration	Time
Dehydration-1	Ethanol	70%	1 h
Dehydration-2	Ethanol	80%	1 h
Dehydration-3	Ethanol	96%	1 h
Dehydration-4	Ethanol	96%	1 h
Dehydration-5	Ethanol	abs.	1 h
Dehydration-6	Ethanol	abs.	1 h
Dehydration-7	Ethanol	abs.	1 h
Intermediate-1	Xylene		1 h
Intermediate-2	Xylene		1 h
Preinfiltration-1	Xylene/Technovit 9100 NEW Basic (Stabilised)	50%	1 h
Preinfiltration-2 (final step when automated)	Technovit 9100 NEW Basic (Stabilised) + Hardener-1		1 h
Preinfiltration-3 (refrigerator)	Technovit 9100 NEW Basic (Destabilised) + Hardener-1		1 h
Preinfiltration (refrigerator)	Technovit 9100 NEW Basic (Destabilised) + Hardener-1 + PMMA-Powder		1 h – 2 or 3 days

New from Leica:

Leica EM TP4C

Automatic tissue embedding machine For resin embedding of mineralised tissue

The Leica EM TP4C enables efficient and standardised resin embedding for light microscope biopsy diagnosis. Fixation, dehydration and resin immersion techniques can be programmed for each individual step regarding time and temperature, including agitation of the samples in the solutions.

- ⇒ Reproducible results from each embedding run
- ⇒ Ensures sample safety
- ⇒ Time and cost efficient
- ⇒ Minimises manual work steps

5. Working with polymerised tissue preparations

5.1. Using a Microtome

- ⇒ Preparation of heavy-duty microtome sections using a bench-top rotary microtome.
- ⇒ As above – but for semi-thin sections using a glass or diamond knife. The blocks must be trimmed before cutting.
- ⇒ Use of 16 mm hardened metal knife with D-form cutting edge or HK3-Knives.
- ⇒ When cutting polymerised Technovit 9100 NEW blocks, 30% ethanol (cutting fluid) must be used.
- ⇒ Transfer sections to superfrost plus slides, mount with 50% ethanol (mounting fluid) and cover with PVC-foil (Kisol-foil).
- ⇒ Remove excess fluid with filter paper. Load the slides into a section-press

5.2. Removal of Polymer from the Sections Prior to Staining – All Steps at Ambient Temperature

- | | | |
|---------------------------------|----------------|------------------|
| ⇒ Xylene | 2 – 3 x 20 min | room temperature |
| ⇒ 2-methoxyethylacetate (2-MEA) | 1 x 20 min | room temperature |
| ⇒ Pure acetone | 2 x 5 min | room temperature |
| ⇒ Aqua Dest | 2 x 2 min | room temperature |
| Alternatively: | | |
| ⇒ 2-MEA | 3 x 20 min | room temperature |

5.3. Cutting-Grinding and Micro-Grinding Systems (Contact Point Technique)

Apparatus for applying these techniques are listed under heading 8.2 below.

6. Routine Staining, Immune Reactions and Enzyme Immunohistochemistry

General Remarks: The following methods for staining of tissues and for detection of signal reactions are given as important examples for processing heavy-duty microtome sections as an introduction to the polymerisation system described in this leaflet. They are analogous to those used with methyl methacrylate (MMA) thin sections.

6.1. Routine Staining

Counterstaining of Sections for Immuno- and Enzyme-Immunohistochemistry

- ⇒ Haematoxylin according to Mayer 30 sec/RT
- Rinse first with tap-water, 10 min/RT
- slowly changing to distilled water
- ⇒ Nuclear Fast Red (C.I. 60760) 10 min/RT
- Rinse under tap water
- ⇒ Methyl Green 10 - 20 min/RT
- Rinse with distilled water

Haematoxylin-Eosin

- ⇒ as for paraffin sections

Giemsa Staining

- ⇒ Remove polymer from sections
- ⇒ Fresh Giemsa solution 30 - 40 min/RT
- Differentiate and Dehydrate
- ⇒ Acetone / Xylene (95 : 5)
- ⇒ Acetone / Xylene (70 : 30)
- ⇒ Acetone / Xylene (30 : 70)
- ⇒ Xylene

RT = room temperature

6.2. Performing the Immune Reaction

Antibody Incubation Step

- ⇒ Rinse the section with 0.01 mol/l phosphate buffer, pH 7.4
- ⇒ Primary antibody 16 h/4 °C or
- diluted in DAKO-antibody diluent 30 - 45 min/37 °C
- ⇒ Rinse with buffer (see above)
- ⇒ DAKO EnVision polyvalent antibody 30 min/RT
- (goat-anti-mouse/goat-anti-rabbit)
- coupled to alkaline phosphatase

Visualisation Step

- ⇒ Rinse with buffer
- ⇒ Chromogenic substrate solution:
- Fast Red TR 15 - 20 min/RT
- ⇒ Counterstain with haematoxylin according to Mayer

RT = room temperature

6.4. Embedding of the sections with DAKO® Ultramount – see fig. 8.2

Masson-Goldner Staining

- ⇒ Remove polymer from sections
- ⇒ Haemalaun according to Mayer 10 min/RT
- ⇒ Rinse under tap water
- ⇒ Ponceau-S-Acid Fuchsin-Azophloxin 45 min/RT
- ⇒ 1% acetic acid
- ⇒ Phosphomolybdicacid / Orange-G (C.I. 16230) 7 min/RT
- ⇒ 1% acetic acid
- ⇒ Light Green SF Yellowish (C.I. 42095) 40 min/RT
- ⇒ 1% acetic acid
- ⇒ increasing concentrations of alcohol
- ⇒ Xylene
- ⇒ Embed in Eukitt (or similar material)

6.3. Enzyme histochemical staining

With Acid- and Alkaline Phosphatase

- ⇒ Rinse sections with 0.1 mol/l 10 min/RT
- Tris buffer, pH 9.4
- ⇒ Incubate in substrate solution 2 h/37 °C
- 0,1 mol/l Tris buffer pH 9,4
- Fast Blue
- Naphthol-AS-BI-phosphate
- ⇒ Rinse with distilled water
- ⇒ Rinse in 0.1 mol/l acetate buffer, pH 5.6 10 min/RT
- ⇒ Incubate in substrate solution 1 h/37 °C
- Hexonium-Pararosaniline solution
- Naphthol-AS-BI-phosphate
- ⇒ Rinse with distilled water
- ⇒ Fix in 40% formalin 2 - 3 h/RT
- ⇒ Rinse with tap water
- ⇒ Counterstain with Methyl Green

With Esterase Reaction using Naphthol-AS-D-chloracetate

- ⇒ Rinse sections with 0.01 mol/l 5 min/RT
- phosphate buffer, pH 7.4
- ⇒ Incubate in substrate solution 1 h/RT
- 0,1 mol/l phosphate buffer, pH 6.5
- Naphthol-AS-D-chloracetate
- Hexonium-Pararosaniline solution
- ⇒ Rinse with distilled water
- ⇒ Counterstain with Haematoxylin according to Mayer.

7. Recipe and Reagents

Buffers and Stock Solutions

SODIUM ACETATE STOCK SOLUTION – 2 mol/l.

74.13 g sodium acetate
5.5 ml glacial acetic acid
make up to 500 ml with distilled water.

SODIUM ACETATE BUFFER – 0.1 mol/l, pH 5.6

50 ml stock solution (see above)
950 ml distilled water adjust pH to 5.6 with either sodium hydroxide (pH too low) or acetic acid (pH too high)

PHOSPHATE STOCK SOLUTION – 1 mol/l

112.5 g disodium hydrogen phosphate
30 g potassium dihydrogen phosphate
make up to 1 litre with distilled water.

PHOSPHATE BUFFER – 0.01 mol/l, pH 7.4

10 ml phosphate stock solution (see above)
980 ml distilled water adjust to pH 7.4 with o-phosphoric acid or sodium hydroxide
make up to 1 litre with distilled water

0.04 mol/l PHOSPHATE BUFFERED 10% SUCROSE – pH 7.4

40 ml phosphate stock solution (see above)
100 g sucrose
1 g sodium azide (e.g. 10 ml 10% NaN₃-solution)
850 ml distilled water adjust pH to 7.4 (see above) and make up to 1 litre with distilled water.

TRIS STOCK SOLUTION – 1 mol/l

121.4 g Tris(hydroxymethyl)aminomethane (Tris)
make up to 1 litre with distilled water.

TRIS BUFFER – 0.1 mol/l, pH 9.4

100 ml Tris stock solution (see above)
850 ml distilled water adjust pH to 9.4 with hydrochloric acid and make up to 1 litre with distilled water.

Stock solutions are best stored in the dark in stoppered brown glass bottles to prevent microbial growth. Diluted buffers can be stored at 4 °C, stock solutions at room temperature.

Fixative Solutions.

BUFFERED FORMALIN SOLUTION (4%)

100 ml 37% formaldehyde (formalin)
4.5 g potassium dihydrogen phosphate
6.5 g disodium hydrogen phosphate
850 ml distilled water.
Adjust the pH to 7.0 with sodium hydroxide or o-phosphoric acid and make up to 1 litre with distilled water.

PARAFORMALDEHYDE STOCK SOLUTION – 8%

40 g paraformaldehyde
make up to 500 ml with distilled water

PARAFORMALDEHYDE SOLUTION – 1.4%

35 ml paraformaldehyde stock solution (see above)
65 ml distilled water
100 ml 0.04 mol/l phosphate buffered 10% sucrose, pH 7.4 (see above)

Reaction Mixtures

FAST RED SOLUTION

3 ml substrate solution
1 Fast Red tablet
120 µl Levamisole
Mix components in a 5 ml stoppered polystyrene or polyethylene test tube. The solution can be then used for approximately 60 min.

ALKALINE PHOSPHATASE SUBSTRATE / REACTION MIXTURE

50 ml Tris buffer – 0.1 mol/l, pH 9.4
50 ml Fast Blue Solution
25 mg Naphthol-AS-BI-phosphate dissolved in 0.5 ml dimethyl sulphoxide (DMSO) / Triton X-100

ACID PHOSPHATASE SUBSTRATE / REACTION MIXTURE

50 ml acetate buffer – 0.1 mol/l, pH 5.6
500 µl Hexonium-Pararosaniline (250 µl Pararosaniline (C.I. 42500) in 2 mol/l hydrochloric acid + 250 µl 4% sodium nitrite in distilled water – Vortex and allow to react for 5 min before use)
25 mg Naphthol-AS-BI-phosphate in DMSO / Triton X-100 (see above)

NON-SPECIFIC ESTERASE SUBSTRATE / REACTION MIXTURE

50 ml phosphate buffer – 0.1 mol/l, pH 6.5
15 mg Naphthol AS-D-chloroacetate in DMSO / Triton X-100 (see above)
250 µl hexonium-pararosaniline (see above)

Staining Solutions

GIEMSA SOLUTION

3 ml Giemsa stock solution (Merck)
97 ml distilled water
1 –2 drops of glacial acetic acid.

LIGHT GREEN

1 g Light Green SF Yellowish
2 ml glacial acetic acid
Make up to 1000 ml with distilled water.

PHOSPHOMOLYBDIC ACID – ORANGE-G

30 g phosphomolybdic acid
20 g Orange-G
Make up to 500 ml with distilled water.

– add both solutions together
– filtrate

PONCEAU-S – FUCHSIN – AZOPHLOXIN

100 ml Masson's solution
20 ml Azophloxin solution
880 ml 0.2 % acetic acid

For Masson's solution mix 1 part of Masson's solution A with 2 parts of Masson's solution B.

Masson's Solution A: 1 g acid fuchsin (fuchsin-S, acid magenta) made up to 100 ml with distilled water heat to boiling add 1 ml glacial acetic acid and filter.

Masson's Solution B: 2g Xylidine Ponceau (Ponceau 2R – C.I. 16150) made up to 200 ml with distilled water heat to boiling add 2 ml glacial acetic acid and filter.

AZOPHLOXIN SOLUTION

0.5 g azophloxin
made up to 100 ml with distilled water and add 2 ml glacial acetic acid.

Signal / Detection Reagents for Immunohistochemistry (IHC) and In-Situ Hybridisation (ISH)

a) Detection System / Second Antibody (IHC) Reagents

EnVision™, goat-anti-mouse or goat-anti-rabbit	Dako	Art. No. K 4017
APAAP – as alternative method:		
rabbit-anti-mouse bridge antibody	Dako	Art. No. Z 0412
goat-anti-mouse bridge antibody	Dako	Art. No. Z 0420
mouse-anti-rabbit second-bridge antibody	Dako	Art. No. M 0737
APAAP-complex – mouse-monoclonal	Dako	Art. No. D 0651
Duet-Kit – Streptavidin-Biotin complex labelled with horseradish peroxidase (HRP) anti-mouse or anti-rabbit	Dako	Art. No. K 0492

b) ISH

Lambda Probe	Bio Genex	Art. No. HK 855-2K
Kappa Probe	Bio Genex	Art. No. HK 856-2K
Detection system for ISH	Bio Genex	Art. No. SH 2008-01
RISH & HRP detection kit (alternative):	KREATCH	Art. No. HKB 27183
Kappa/Lambda Light Chain RNAs (DAKO), Biotin labelled		

c) Additional Reagents

DAKO Antibody diluent	DAKO	Art. No. S 0809
Fast-Red-Solution	DAKO	Art. No. K 0699
Ultramount Embedding Solution	DAKO	Art. No. S 1964

8. Delivery Units and Accessories

8.1 Delivery Units from Heraeus Kulzer

- Technovit 9100 NEW Combi-Pack	Art. No. 64715444
- Teflon embedding mould	Art. No. 64711225
- Histoblocks	Art. No. 64712817
- Polyethylene foil	Art. No. 64712818
- Slide press	Art. No. 64712819

8.2 Addition Apparatus and Suppliers

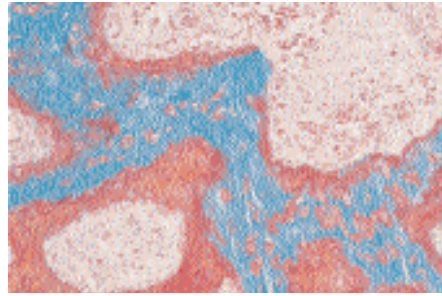
The methods described in this booklet using Technovit® 9100 NEW have been carried out with standard laboratory equipment, together with special materials and equipment from the following suppliers:

EBS temperature regulator	Fa. Elektronik-Bau, K. Schneider, D-18442 Groß-Lüdershagen, Telefon: +49-3831-270250
Microtomes for grinding and microgrinding	Fa. Exakt Apparatebau GmbH & Co KG, D-22851 Norderstedt, Telefon: +49-40-529560-0
Reagents for Immunochemistry	Fa. DAKO D-22047 Hamburg, Telefon: +49-40-6969470

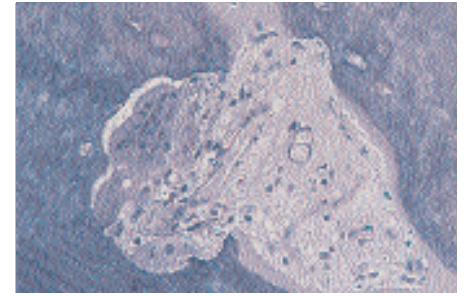
9. Photomicrographs



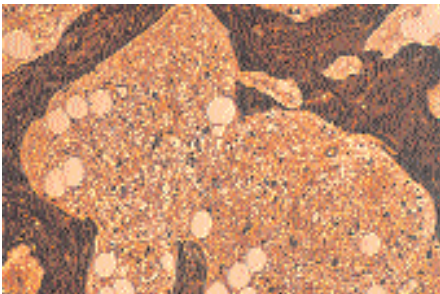
Pelvic biopsy: Renal osteopathy
Masson-Goldner
Deep Howship's Lacunae with osteoclasts
400x



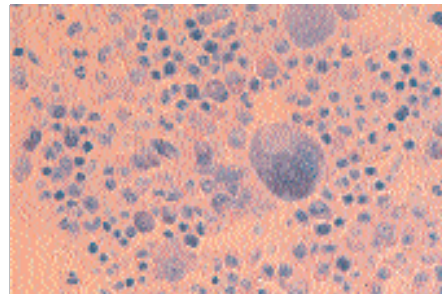
Pelvic biopsy: Osteomalacia intestinal form
Masson-Goldner
osteoid tissue red; mineralised bone green
400x



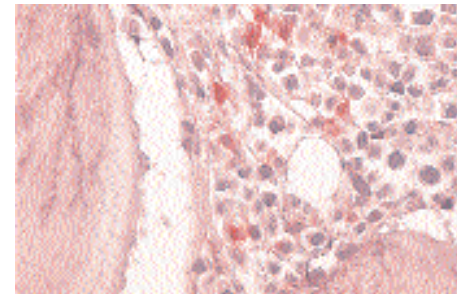
Pelvic biopsy: Toluidine Blue
Paget's Disease with giant osteoclasts
200x



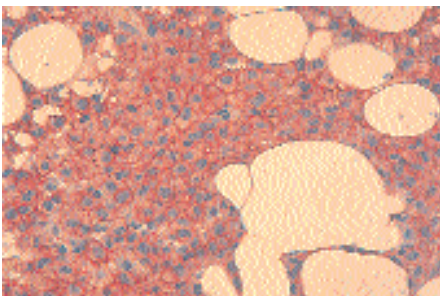
Pelvic biopsy: Myeloproliferative syndrome
presenting as osteomyelofibrosis
Gomori
200x



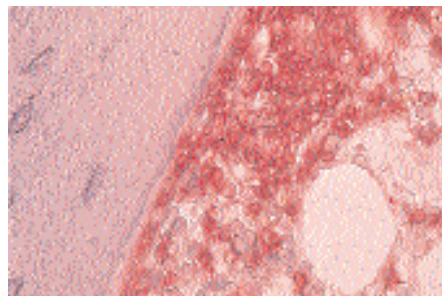
Pelvic biopsy:
Reactive hyperplastic bone marrow
Giemsa
400x



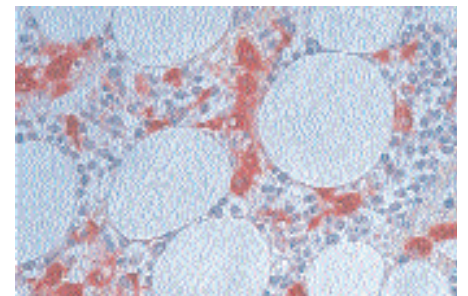
Pelvic biopsy:
Reactive hyperplastic bone marrow
immunohistochemistry CD 45 RB
(DAKO; APAAP) 400x



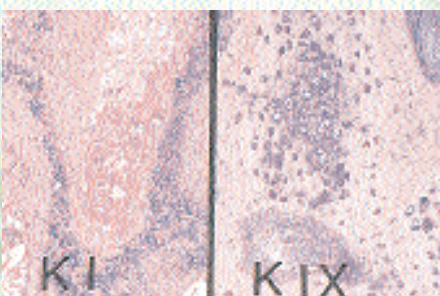
Pelvic biopsy: Hair-cell leukaemia
immunohistochemistry CD 20 (DAKO; APAAP)
400x



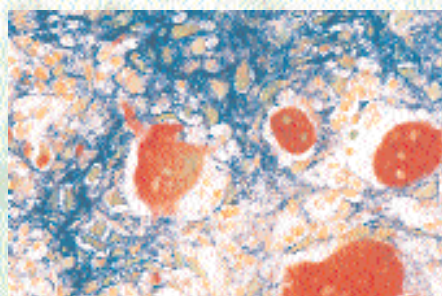
Pelvic biopsy:
Mantle-cell lymphoma or centrocytoma
para-trabecular infiltration
immunohistochemistry CD 5 (DAKO EnVision)
400x



Pelvic biopsy: Situation after chemotherapy
mamma carcinoma – activated reticulum cells
KiM 1P histochemistry CD 68 (DAKO EnVision)
400x



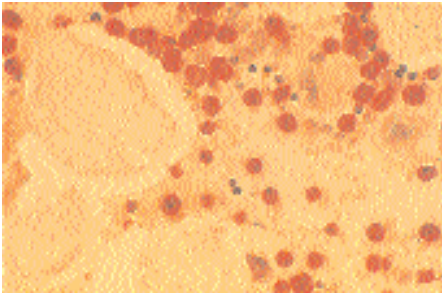
Bone tumour: Central chondrosarcoma
staining of type I (K-I) and type IX (K-IX) collagens
Thin section after EDTA-decalcification. APAAP;
Fast Blue salt; Counterstain
Nuclear Fast Red – 200x



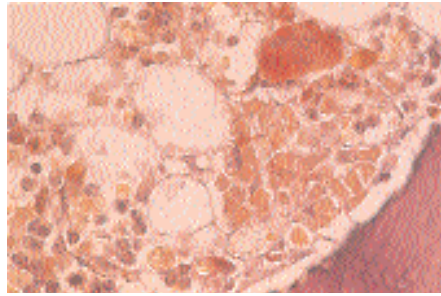
Giant Cell Tumour: Enzyme histochemistry:
Combined demonstration of alkaline (blue) and
acid (red) phosphatases. Nuclear counterstain
methyl green
400x



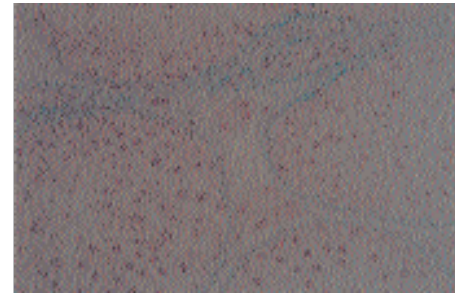
Pelvic biopsy: Enzyme histochemistry:
Physiological resorption with shallow Howship's
Lacunae. Demonstration of acid phosphatase
(red) in singly nucleated osteoclasts
400x



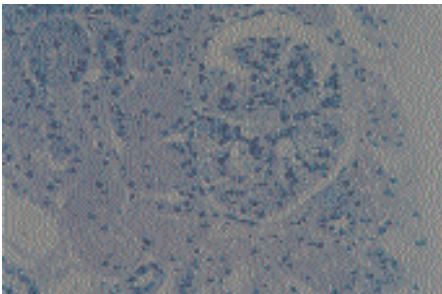
Pelvic biopsy: Enzyme histochemistry; combined demonstration of specific esterase (naphthyl-AS-D-chloroacetate) in granulopoietic cells
400x



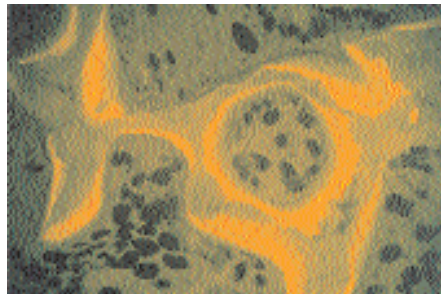
Pelvic biopsy: Demonstration of non-specific esterase (neutral α -naphthyl acetate esterase). Reaction product red-brown precipitate in megakaryocytes and monocytes



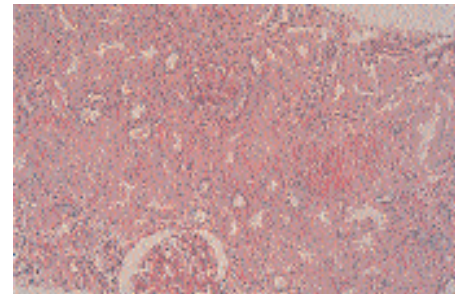
Testicular biopsy: semi-thin section normal spermatogenesis fixation according to Masson-Goldner
400x



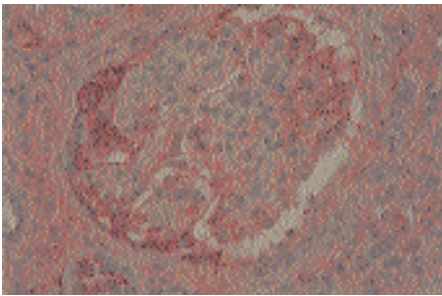
Renal biopsy: Thin section Toluidine blue idiopathic nephritic syndrome with slight glomerular abnormalities (minimal glomerular cellularity)
400x



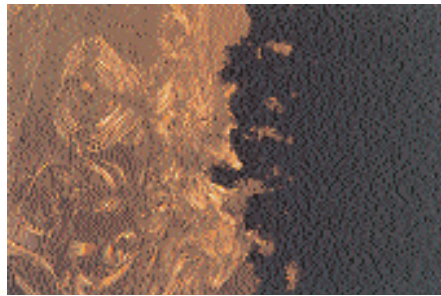
Pelvic biopsy: Osteomalacia with tetracycline labelling non-stained thin section under ultra-violet light
200x



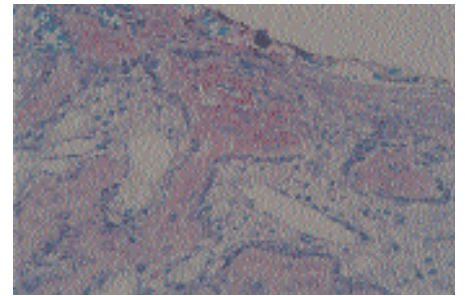
Renal biopsy: Thin section diffuse mesangioproliferative and sclerotic glomerulonephritis periodic acid-Schiff (PAS)
200x



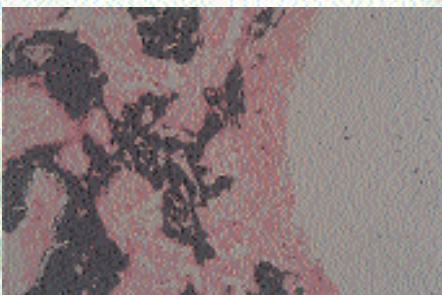
Renal biopsy: Thin section Immunohistology IgG DAKO segmented sub-endothelial mesangial deposits of immune complexes APAAP
600x



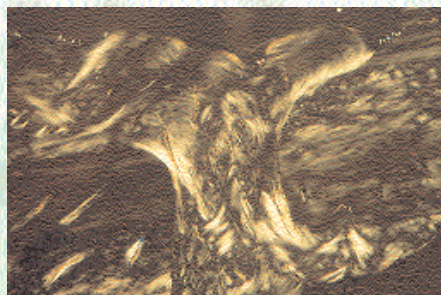
Micro-ground section: Bone implant contact zone bone-metal contact zone in polarised light
400x



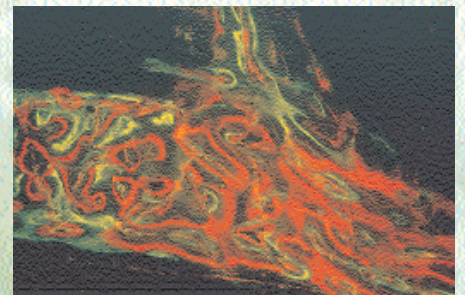
Hard section: After removal of implant Border area with fibrous bone and activated osteoblasts Giemsa
400x



Hard section: After removal of implant Border area with fibrous bone and activated osteoblasts Kossa. Mineralisation of fibrous bone
400x



Thin section: Femur after osteotomy (rat). Osteotomy area in polarised light fibrous and lamellar bone in osteotomy cleft unstained
200x



Thin section in UV-light: Polychromatic sequential labelling for the monitoring of dynamic bone healing in cleft and at the level of the periosteal fracture callous
400x

Components of the Technovit 9100 NEW Polymerisation System

Component	Amount	Component Number
Basic solution (stabilised)	1 litre	1
PMMA-Powder	120 g	2
Hardener 1	8 Sachets á 1 g	3
Hardener 2	10 ml	4
Polymerisation regulator	5 ml	5
PMMA-Granulate, EXAKT*	500 g	6

* EXAKT: Trade Mark of EXAKT-Apparatebau GmbH & Co. KG, D-22851 Norderstedt

Preparation of a ready-to-use solution from the Technovit 9100 NEW - Components 1 - 5

Component no.	1	2	3	4	5	
Component	Basic solution	PMMA-Powder	Hardener 1	Hardener 2	Polymerisation regulator	Storage
Pre infiltration	200 ml		1 g			Room temperature
Infiltration	ad 250 ml	20 g	1 g / 2 g*			4 °C
Stock solution A	ad 500 ml	80 g	3 g / 4 g*			4 °C
Stock solution B	ad 50 ml			4 ml	2 ml	4 °C

Important: *When using stabilised Technovit 9100 NEW the increased amounts of Hardener 1 must be used.

Dehydration, Intermediate and Immersion (Preinfiltration steps 1-3, Infiltration)

Step	Solution	Concentration	Time (h)
Dehydration-1	Ethanol	70%	1
Dehydration-2	Ethanol	80%	1
Dehydration-3	Ethanol	96%	1
Dehydration-4	Ethanol	96%	1
Dehydration-5	Ethanol	abs.	1
Dehydration-6	Ethanol	abs.	1
Dehydration-7	Ethanol	abs.	1
Intermediate-1	Xylene		1
Intermediate-2	Xylene		1
Preinfiltration-1	Xylene/Technovit 9100 NEW Basic (Stabilised)	50%	1
Preinfiltration-2 (final step when automated)	Technovit 9100 NEW Basic (Stabilised) + Hardener-1		1
Preinfiltration-3 (Refrigerator)	Technovit 9100 NEW Basic (Destabilised) + Hardener-1		1
Infiltration (Refrigerator)	Technovit 9100 NEW Basic (Destabilised) + Hardener-1 + PMMA-Powder		1 – 2 or 3 days

References and Production Notes

For complete methodology and adaptation of methods to MMA-sections and thin sections refer to: K. Donath, K. Röser and E. Wolf – Laborleitfaden zum Technovit 9100 NEW. – Einbettungssystem. Available July 2001.

Further information can be obtained from the companies listed in section 8 above.

Development, scientific advice and photomicrographic pictures using Technovit 9100 NEW have been produced in conjunction with Heraeus Kulzer.

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