Technical Data Sheet

Technovit® 9100 Methyl Methacrylate Applications

#14655

Plastic Embedding System for Medicine, Botany and Zoology

Technovit® 9100 was specifically developed for the embedding of mineralized 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 hybridization.

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

Fields of application:

- Hard-cutting technique for making thin layers
  Examples: Iliac 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-bearng (metal or ceramic) jaw or long bones, or brittle, hypermineralized bones.

Material properties

Polymerization 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 polymerization in the cold (in the range of -2 to -20°C, depending on the volume) that guarantees complete dissipation of the polymerization heat.

The benefits of the system at a glance:

- Polymerization below freezing

- Reproducibility of the embedding results and reliability 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 hydrophilizing agent
- Can be used for thin section and the sawing and cutting techniques
- Enzyme histology and immunohistology as well as in-situ hybridization possible (sections)

Product Data

Designation	**Quantity**
Technovit® 9100	1 x 1000 ml Basic Solution 1 x 120g PMMA Powder 8 x 1g Hardener 1 1 x 10 ml Hardener 2 1 x 5 ml Regulator
Technovit® 9100 Basic Solution	5000 ml
Technovit® 9100 PMMA Powder	1000g
Technovit® 9100 Hardener 1	100 x 1g
Technovit® 9100 Hardener 2	9 x 10 ml
Technovit® 9100 Regulator	12 x 5 ml

The Components

- Technovit® 9100 Basic Solution - Component 1
  The Technovit® 9100 basic solution is comprised of stabilized methyl methacrylate. The hydrophily is improved through the addition of a suitable hydrolyzing agent. Technovit® 9100 basic solution can be used when stabilized and unstabilized.
- Technovit® 9100 PMMA Powder - Component 2
  The PMMA powder is used to guarantee a clear decrease in polymerization shrinkage, a reduction in the polymerization heat released and a better polymerization process.
- Technovit® 9100 Hardener 1 - Component 3
  Hardening powder 1 is a peroxide compound that starts polymerization with hardener 2.
- Technovit® 9100 Hardener 2 - Component 4
  Hardening liquid 2 acts as a catalyst for hardener 1 to facilitate targeted polymerization even at very low temperatures [< 0°C].
- Technovit® 9100 Regulator - Component 5
  This is comprised of a reactive organic compound that facilitates a regulated polymerization with controlled low temperature spikes even for large quantities of polymerization.

- PMMA-Granulate, EXART®
  This granulate acts as an additional internal filler when larger amounts (500-1000 ml) of polymer are to be used, for example, in the case of femur shaft with noncemented endoprosthses. The amount of monomer (basic solution) is thereby reduced, at the same time making the polymerization easier to control.

Designation	Quantity	Component Number
Technovit® 9100 Basic Solution Stabilized	1 x 1000 ml	1
Technovit® 9100 PMMA Powder	120g	2
Technovit® 9100 Hardener 1	8 bags, each 1g	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 iliac 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)

NOTE: 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 iliac crest biopsies (the times and volume must be adjusted for larger tissue specimens).

Dehydration, intermedium and pre-infiltration			
Phase	**Solution**	**Concentration**	**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 Basic (stab.)		>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 polymerization solution is thereby reduced.

Destabilization ofthe Basis Solution - processing the components

Technovit® 9100 basic solution can be used when stabilized and unstabilized. The application of destabilized basic solution guarantees that the results for all immunohistochemical studies are analagous to the paraffin histology. Fill chromatography column with approx. 50g 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 destabilized 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. Destabilized basic solution can be worked with starting with pre-infiltration 3. When working with

destabilized 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!
- Polymerization 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 Number	**1**	**2**	**3**	**4**	**5**		
Designation	**Basic Solution**	**PMMA Powder**	**Hardener 1**	**Hardener 2**	**Polymerization/ regulator**	**Processing temperature**	**Storage and shelf life**
Pre-infiltration 3	200 ml		1g			Room temp	1/2 year at -20°C
Infiltration	ad* 250 ml	20g	1g / 2g**			4°C	1/2 year at -20°C
Stock solution A	ad* 500 ml	80g	3g / 4g**			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 stablized Technovit® 9100 NEW the greater amount of hardener 1 must be used.

Polymerization

The polymerization mixture is poured into the pre-cooled embedding form. Place the infiltrated tissue into the form, pour the polymerization 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!

Polymerization 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.

Polymerization is complete in approximately 24 hours. The polymerization times depend on the polymerization 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 polymerization!

Blocking and archiving

Once the specimens have warmed to room temperature after hardening, use Histoform N to block with Histobloc® and Technovit® 3040. 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 polymerized 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) overnight 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.	RT

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

Division thin section technique

Division with point contact technology and cutting with surface contact or line contact processes with corresponding devices.

Technical Data	
Color	Transparent
Density = spez. weight g/cm^3 (DIN 53479)	1,07
Refractive Index Monomer Polymer	 1,4175 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
- Vacuum pump
- Magnetic stirrer

Source of Information

Heraeus Kulzer, 2014