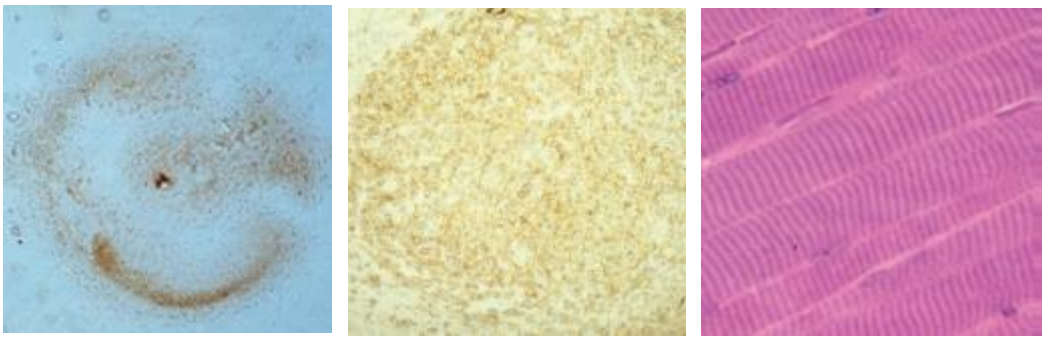
TECHNOVIT® 8100
SECTIONS SPECIFICALLY FOR IMMUNOHISTOCHEMISTRY
Product No. 813-800

Acquired from Kulzer Mitsui Chemicals Group, Technovit-Histology, Polymerization Systems for Histological Application

DESCRIPTION AND USES

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



Sections with Technovit® 8100 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 odorless plasticizer and a hydrophilic plastic. Technovit® 8100 was specifically developed for cold polymerization (+4°C).

While hardening, the embedding mold must be hermetically sealed because the polymerization 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 polymerization temperature of 10°C to 0°C due to the special catalyst system and the PTFE molds
- Uniform hardening of the block, thus uniform and thinnest possible sections
- Low shrinkage artefacts, thus excellent tissue morphology
- Routine staining, enzyme detection and immunohistochemistry possible
- Hematological iliac crest biopsies do not need to be decalcified
- Low toxicity due to special combination of plasticizer and catalyst system

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APPLICATION

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

POLYMERIZATION

Prepare the polymerization 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 polymerization is complete and blocked with Histobloc® and Technovit® 3040.
It is not possible to elute the plastic before staining or reaction.

TECHNICAL DATA	
Color	Transparent
Density = spec. weight g/cm3 (DIN 53479)	1.08
Refractive index Monomer Polymer	 1.4485 1.4990
Storage temperature	Max. 25 °C
Shelf life	2 years

The following instructions for fixation and dehydration are not necessarily required. Technovit® can also be infiltrated and polymerized 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.
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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 in the infiltration solution for 6-10 hours at 4°C.

Polymerization

Making the polymerization solution:

- Infiltration solution, 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 color of the polymerization solution changes first to yellow-green, but after hardening it becomes colorless. Completely fill the Histoform embedding molds with a disposable pipette, position the tissue therein and immediately.

MAKING THE SOLUTION						
Solution	Ethanol	Basic solution	Hardener 1 Technovit® 8100	Infiltration Technovit® 8100	Hardener 2 solution	Application Technovit® 8100 temperature.
Infiltration		100 ml	0.6 g (1 bag)			4 °C
Polymerization				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 polymerization solution and add new film. During polymerization (at least 3 hours), the embedding mold must be placed on a cooling plate or thin layer of ice at 4°C. Do not let the mold 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

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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 mold. 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 rotary microtome, with the Technovit® Histo blade in combination with the Kulzer knife holder or a hard metal knife, a glass knife, or diamond knife. Tightly clamp the blocks in the totem cam system on the microtome. Dryly remove the sections with forceps and place in a water bath. Place 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 and 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 coat the object holder with 0.1% poly-L-lysine (#18026)
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 sections 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 peroxide 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 cryostat sections
- 10-15 seconds of counterstaining with hematoxylin
- Blue for three minutes under flowing water
- Cover with glycerin gelatin

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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, it is always advisable to follow the respective manufacturer instructions.

SOURCE DOCUMENT

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