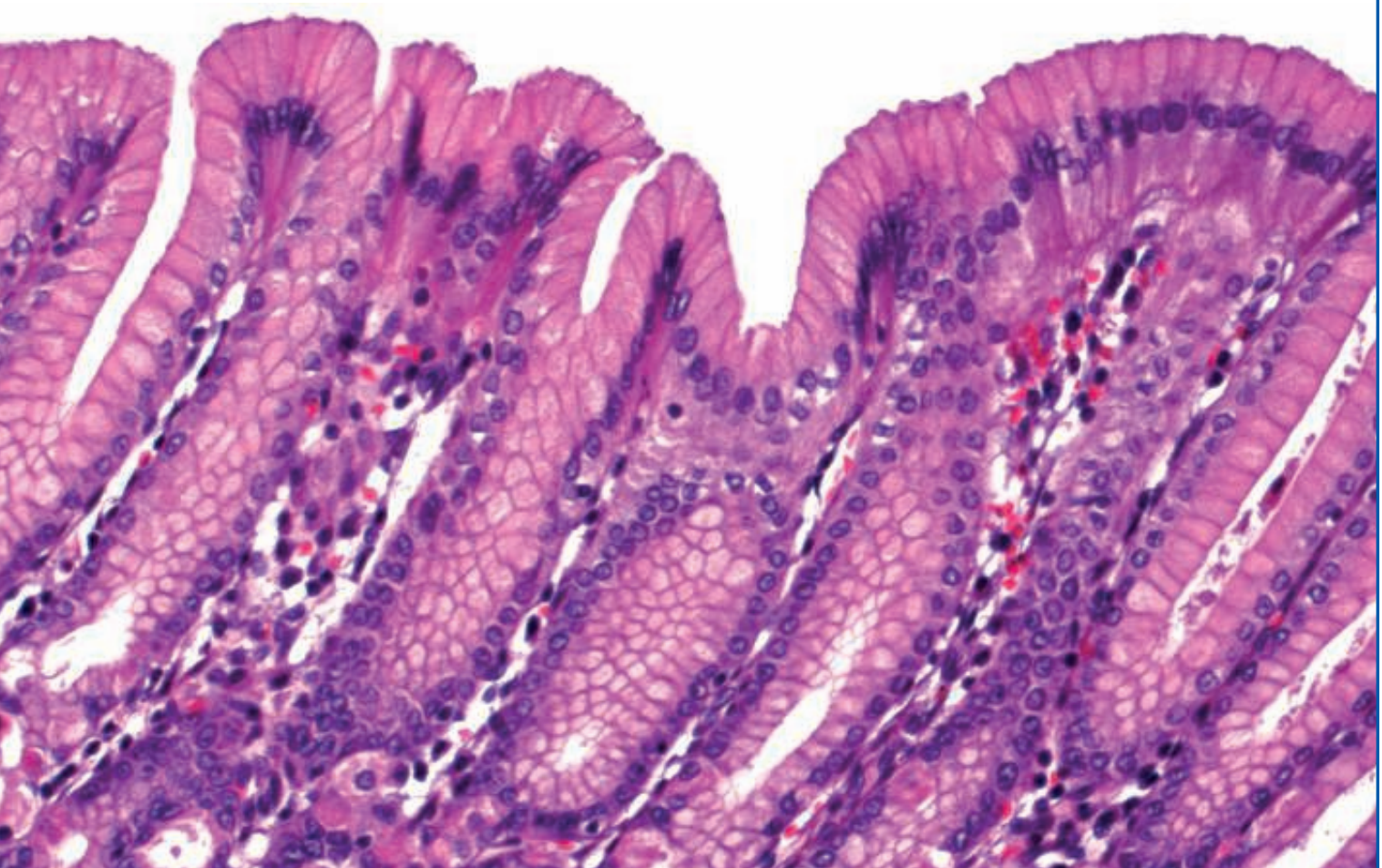
MERCK MILLIPORE

Fast and reliable.
All you need for histology.

Merck Millipore is a division of MERCK



Content

Introduction

CE certification of IVDs

The European In Vitro Medical Devices Directive (98/79/EC) of 20 October 1998 is the legislative instrument on which local IVD legislation within the EU Member States is based. It has been mandatory since December 2003 for all In Vitro Medical Devices (IDVs) that are placed on the market in the EU by manufacturers to be CE certified and bear the CE marking on product labels, instructions for use and sales packaging. All IVDs placed on the market by suppliers and used as IVDs must bear the CE marking as from December 2005.

The term In Vitro Diagnostic Devices means any medical device which is a reagent, reagent product, calibrator, control material, kit, instrument, apparatus, equipment or system, whether used alone or in combination, intended by the manufacturer to be used in vitro for the examination of specimens, including blood and tissue donations, derived from the human body, solely or principally for the purpose of providing information:

- concerning a physiological or pathological state,
- concerning a congenital abnormality,
- to determine the safety and compatibility with potential recipients,
- to monitor therapeutic measures

The Directive groups IVDs into categories as follows:
Class 2 – IVDs in Annex II List A (which includes test kits for HIV and some blood grouping products) and List B (which includes test kits for various antibodies and viruses and test kits for blood glucose).
Class 1a – IVDs for self testing, other than those in Class 2.
Class 1 – all IVDs other than those covered by Annex II and IVDs for self testing. Our "Products for Microscopy" belong to Class 1.

CE certification is a self-certification process in which product details are submitted according to the valid EDMA (European Diagnostic Manufacturers Association) code based on the [in Germany] VDGH (Verband der Diagnostika-Industrie e.V.) classification. Merck successfully completed the CE certification process in December 2003 for all Products for Microscopy at the time that were used as IVDs and belonged to the low-risk group. New products are developed, manufactured, quality-tested and documented in accordance with the regulations, and are registered with the Competent Authority before being placed on the market. The CE certification process also covers the conducting of regular in-house and external quality management system audits in accordance with ISO EN 13485.

Fixatives

Fixing a specimen means interrupting the complex intravital and supravital metabolic processes that occur in tissue, preserving structures and preventing postmortal decay. Fixation fundamentally changes the native state of the tissue and any examinations are based on an image that is characteristic of the fixative used. In histology the specimen material is saturated in liquid fixative, then finely sliced and immersed in fixative of volume at a minimum of 20 times greater than the volume of the tissue to be fixed.

Your benefits

- Autolysis, caused by the cells' own enzymes
- Putrefaction, caused by microorganisms
- Decomposition, under atmospheric oxygen
- Rotting, same process but without oxygen

Purpose of fixing

Proteins, soluble organic substances, are denatured with formaldehyde to render them insoluble and biologically inactive and to induce crosslinking. Reacting the protein side chains with formaldehyde leads to crosslinking, which enables antigens to be masked and incapable of reacting with antibodies unless specially pretreated.

$$RH + CH_2O \rightleftharpoons R\text{-}CH_2(OH)$$

$$R\text{-}CH_2(OH) + HR' \rightleftharpoons R\text{-}CH_2\text{-}R' + H_2O$$

Lipids are fixed and stabilized through fixation of the protein fraction. The lipids are leached out with alcohol and are visible as vacuoles or empty mesh-like structures. Carbohydrates are stabilized through the fixed surrounding tissue. Nucleic acids lose their structure when fixed and form aggregates. Stabilization is due to the fixed surrounding tissue. Intercellular spaces become larger. Boundaries between various structures may exhibit massive structural damage. Structures containing water may exhibit osmosis-induced shrinkage or swelling.

Formaldehyde

Formaldehyde (HCOH) is one of the most familiar and effective fixatives. It has been used for over 100 years. Its aqueous solutions are known as formalin or formol.

Advantages of formaldehyde fixing

Structures are maintained. The stock solution is an aqueous approx. 40 % solution of formaldehyde. Working solutions are 4 % or 10 % solutions. Fixed specimens remain stable for a long time; the material hardens up, making it easy to work with. Enzymes such as alkaline phosphatase, unspecific esterases, specific esterase and lipase remain active following formalin fixing.

When kept in dilute formalin solutions, specimens can be stored practically indefinitely. Formalin, even in very low concentrations, has a disinfecting effect on viruses, bacteria, spores, tubercle bacteria, hepatitis and AIDS viruses.

Disadvantages of formaldehyde fixing

Histochemical investigations may become difficult when enzymes and epitopes are masked. Masking can be partially eliminated, however, by washing. Formaldehyde can cause health problems, is allergizing and is a chemical irritant.

Technical information

Formaldehyde is a rapid fixative that perfuses approximately 1 mm of tissue per hour. Fixing times should not be shorter than 8 hours to allow complete fixation of the specimen. Keeping specimens in the cold can lead to the formation of paraformaldehyde as a result of polymerization; this settles out as a white precipitate, so reducing the concentration of the solution. It is important, therefore, not to keep solutions in the refrigerator. Formic acid formed, for instance, through the action of light in nonstabilized and unbuffered solutions can impair nuclear staining. Formaldehyde fixation is particularly suitable for the following types of specimen material: overview specimens, calcified deposits, bone, cartilage, nervous system. Acidic formaldehyde solutions impact negatively on silver stains, so the pH should remain in the neutral range. Other fixatives may be used in addition to formaldehyde. When working with formaldehyde solutions it is vital to ensure that the workplace is well ventilated. The 4 % working solution is adjusted with phosphate buffer to protect tissue structures. The solution is suitable for all types of tissue and ensures effective and, at the same time, gentle fixation.

The 37 % stock solution is protected against shifts of pH into the acidic range through the addition of calcium carbonate. 10 % methanol affords effective protection to stop formaldehyde being polymerized to paraformaldehyde.

Ordering information

Name	Packaging	Ord. No.
Formaldehyde solution 4 % buffered	5 l, 10 l Titripak®	100496
Formaldehyde solution min. 37 % free from acid, stabilized with about 10 % methanol and calcium carbonate	1 l, 2.5 l	103999

Other fixatives

The literature cites a number of other fixative mixtures featuring chemicals that are no longer available for this purpose, such as sublimate, picric acid, chloroform, chromic acid and uranyl acetate. The benefits of these fixative mixtures in the visualization of structural peculiarities are more than cancelled out by the toxic and hazardous nature of the reagents, to which users and the environment are no longer allowed to be exposed.

Ethanol

Ethanol (ethyl alcohol) with a content of 96 % or 100 % (absolute) is another frequently used fixative. It works by extracting water from specimen materials without affecting or altering their structures or chemical constituents. The denaturant used to denature the alcohol is methyl ethyl ketone which behaves neutrally in the applications for which it is used. The specimens to be fixed should not be larger than 5 mm. 96 % or 100 % ethanol leads to rapid fixation, taking between 30 and 60 min for specimens that are 1-2 mm thick or between 2 and 4 hours for those with a thickness of 3-4 mm. The rapid dehydration caused by ethanol fixation often leaves the specimen hard and brittle and not always easy to section. It can happen that the specimen is fixed and hard on the outside, while inside it is still not properly fixed. This can be avoided by starting fixation using ethanol solutions of low concentration such as 50 % to 70 %. Shrinkage can be avoided by fixing in cold solutions.

Ordering information

Name	Packaging	Ord. No.
Ethanol absolute, for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 l, 2.5 l	100983
Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 l, 5 l	100974

Returnable steeldrum with withdrawal system for inert gas-pressurizing

Decalcification

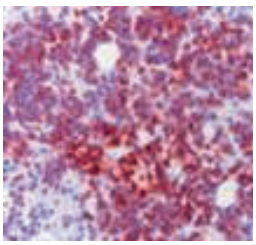
OSTEOMOLL® and OSTEOSOFT®

Decalcification methods for optical microscopic examinations of bone and other hard tissue in routine histological procedures

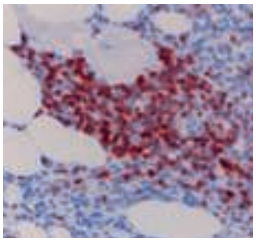
For sensitive tissues such as punched iliac crest specimens, which must be gently decalcified to preserve cell and antigen structures and to allow immunohistochemical methods to be applied following decalcification, a gentle decalcifying solution such as OSTEOSOFT®, Ord. No. 101728, is recommended.

For more robust bone material a bone decalcifying solution that allows fast, reliable decalcification should be used: e.g. OSTEOMOLL®, Ord. No. 101736. OSTEOMOLL® is an acidic formaldehyde-containing decalcifying solution, so that decalcification and fixing occur in a single step. OSTEOMOLL® decalcifies bones in a time that ranges, depending on size, weight and density, from 30 minutes to a maximum of 8 hours, e.g. for hip bone fragments. This means that procedures for processing hard material have to be checked as the stainability of the material may be reduced if treated too long with OSTEOMOLL®.

If the decalcified material is found to be still too hard for sectioning, the entire block can be placed for a few minutes, specimen side down, in a flat dish containing OSTEOMOLL® to allow further decalcification to occur. If the material persists in being too hard, the procedure may be repeated. Dry the paraffin block carefully and section as usual.



Bone marrow biopsy, paraffin section, OSTEOSOFT®, immunohistochemistry, Plasmocytome



Bone marrow biopsy, paraffin section, OSTEOSOFT®, immunohistochemistry, reactive changes

If hard material like bones or teeth is to be histologically processed, it must either be decalcified for processing by the standard paraffin embedding and paraffin slicing procedure or it must be plastic-embedded for microtome sectioning.

Ordering information

Name	Packaging	Ord. No.
OSTEOSOFT® Bone decalcifying solution for sensitive calcium-containing tissue	1 l, 10 l	101728
OSTEOMOLL® Bone decalcifying solution for bone and hard tissue	1 l, 2.5 l	101736

Histoprocessing

Paraffin for histoprocessing and blocking

Paraffins are mixtures of saturated hydrocarbons that have a melting point that increases with chain length. Paraffins used in histology have chain lengths of between 20 and 35 carbon atoms (and corresponding melting points of between 38 °C and 70 °C). The consistency of the solidified paraffin in turn increases with the melting point. For routine histological work a paraffin with a melting range of 56-58 °C is used. Paraffin crystallizes as it solidifies. With rapid cooling the solidified paraffin assumes a fine homogeneous crystalline structure and is easy to slice. When cooled only gradually the paraffin crystallizes to form irregular plates and needles and suffers shrinkage. The surface of the solidifying paraffin block becomes highly indented and air-filled gaps can form between the paraffin crystals. The latter are seen as a mottling of the paraffin, which normally has a glassy transparent appearance. The mottling is disruptive during slicing, as the slices lose their integrity at these points and break off. Various substances are added to the paraffin to prevent this effect; the classic additive is 5 % beeswax. Modern paraffins contain plastic polymers to prevent mottling, improve sectioning and increase the rate of infiltration. Paraffin should not be heated to more than 2-3 °C above its melting point. Paraffin that has been overheated oxidizes to a yellow color and saponifies when cooled.

Addition of DMSO (dimethyl sulfoxide) makes for faster and complete penetration, while addition of polymers lends greater plasticity to embedded tissues. The high degree of purity makes it unnecessary to filter the molten wax. Excellent slicing properties are assured for individual and multiple slices and the form and color of the histological stain are preserved.

Histosec®

First class quality paraffin for routine use, immunohistochemical staining and microwave applications

Histosec®

Histosec® is a high-purity paraffin that is available with and without DMSO. Its melting range is between 56 and 58 °C and, within this range, any specimen can be processed without fear of damage irrespective of the method used.

For optimized and more comfortable serial ribbon cutting in hot summer periods was created Histosec® S with a melting range between 58 and 60 °C.

Histosec® is produced in a complex process from carefully selected raw waxes and other starting materials. lt undergoes rigorous quality controls.

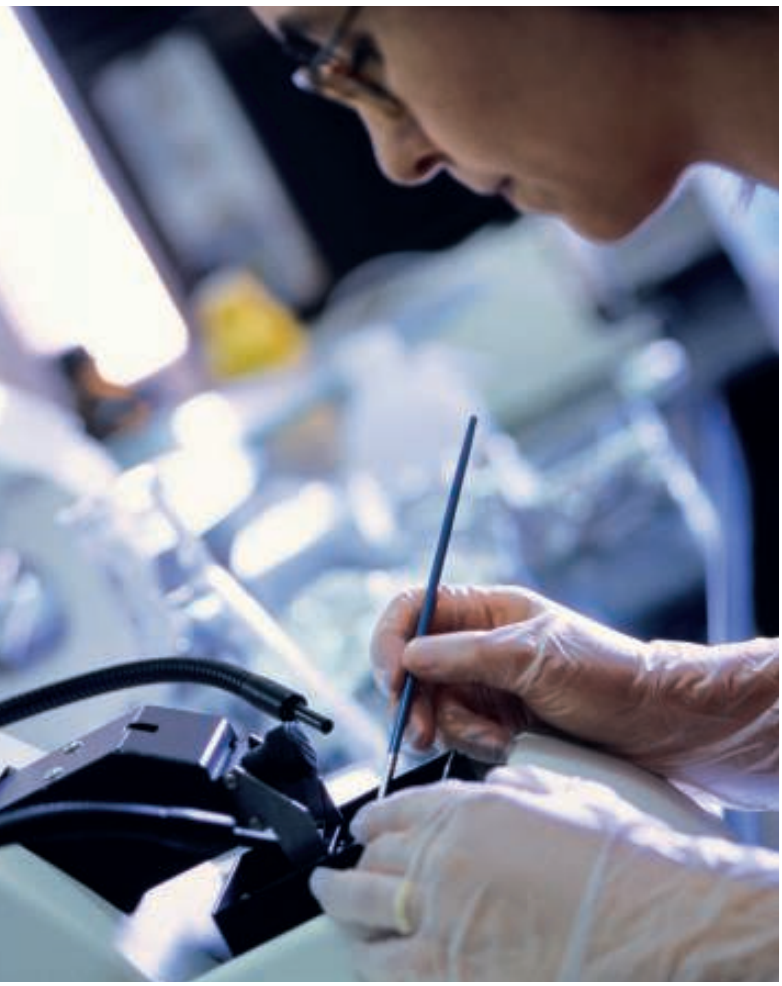
In histoprocessing, to achieve total dehydration the specimens are fixed and rinsed and then passed through ascending concentrations of alcohol, then through baths containing intermedia, i.e. solutions that are miscible with alcohol as well as with paraffin. The intermedia mostly used today are xylene and xylene substitute. After all of the water has been removed, the specimens are placed in paraffin baths and perfused with paraffin. At the end of the process, paraffin will have penetrated points where water or alcohol-soluble substances such as fat were previously located in the native material. Notable features of Histosec® are its excellent lifetime in the histoprocessor and outstanding stability throughout its use.

Histoprocessing protocol

Product	Concentration	Time
Ethanol	50 %	1 h
Ethanol	70 %	1 h
Ethanol	70 %	1 h
Ethanol	80 %	1 h
Ethanol	90 %	1 h
Ethanol	100 %, denatured	1 h
Ethanol	100 %, denatured	1 h
Ethanol	100 %, denatured	1 h
Xylene or Neo-Clear®		1 h
Xylene or Neo-Clear®		1 h
Histosec®	60 °C	2 h
Histosec®	60 °C	3 h

Histosec®
products are selected paraffins
with added polymers

To assure easy preparation of multiple sections, the dehydrated and paraffin-perfused specimens are blocked in paraffin, then cooled and kept in a cool place.



Technical information

If technical grade paraffin is used or if paraffin is used for too long in the histoprocessor, it may absorb too much intermedium. The appearance of the paraffin changes; it becomes greasy, smells of the solvent and is unable to penetrate the specimens as effectively. As a consequence, the specimens may not be completely penetrated and may be more difficult to slice afterwards. Freshly blocked specimens in particular can cause problems and the quality of the slices is poorer.

Ordering information

Name	Packaging	Ord. No.
Histosec®	1 kg, 4 x 2.5 kg, 25 kg	111609
Histosec® without DMSO	4 x 2.5 kg, 25 kg	115161
Histosec® S	4 x 2.5 kg	101676
Pure paraffin		
Paraffin	4 x 2.5 kg	107164

Paraffin sections

For some staining procedures, such as congo red staining to detect amyloid, the sections should be 7 µm thick; for detecting mycobacteria by the Ziehl-Neelsen method the thickness should be at least 5 µm to enable the target structures to be properly stained.
Difficulties in preparing slices are experienced when the paraffin blocks are too soft, too hard or brittle, or when the paraffin still contains solvent residues from histoprocessing. Stable and reproducible results can only be achieved by using top-grade solvents, a paraffin that has been specially tested for use in histology and an appropriate protocol. Using the protocol, solvents and paraffins described here leads to excellent, reproducible results and eliminates unnecessary costs and disruptions during preparation of the specimen material.

Slicing
Paraffin sections are prepared using either a sled or a rotary microtome. In both cases the blade moves horizontally over the block of embedded specimen. In the rotary microtome linear motion is created with a hand-operated flywheel and transformed into a rotary motion. Details of the particular microtome used can be found in the instructions for use. Classical microtome blades designed for repeated use can be used in both device types. Disposable blades are suitable for use in rotary microtomes. Blocks should always be kept cool prior to slicing.
The microtome is set to the desired slice thickness, which for normal specimens will be about 3-5 µm, and the sections will be produced in that thickness with the blade moving evenly over the restrained paraffin block. The sections are removed from the blade with a fine brush and are ready for further processing.

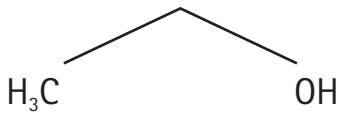
Attaching and stretching
The sections are stretched by placing in a warm water bath and from there placed on a clean microscope slide. For special applications the microscope slides are coated, or commercially pre-treated microscope slides are used, so as to stop the sections becoming detached during subsequent processing. After the sections have been attached, the microscope slides are placed in a heating cabinet to dry.

Preparing for deparaffination
The paraffin sections are melted for about 20 minutes at approx. 70 °C in a heating cabinet and further processed as directed in the protocol.

Solvents

Ethanol

Ethanol, C_2H_5OH, is among the most frequently used solvents in histology. It is used in histoprocessing, for the ascending and descending alcohol series employed in staining, for rinsing during staining as well as in numerous staining solutions. The application instructions explain how ethanol is used for histoprocessing and staining. Ethanol acts swiftly and effectively. Baths must be exchanged often to prevent loss of quality seen as decolored sections or milky-turbid slides.



Being flammable, ethanol bears the F hazard symbol. Safety instructions and other information on how to handle ethanol without risk are contained in the safety data sheet which is available via the Web or upon request.

Ethanol is a solvent having the following physicochemical properties

Chemical and physical data			
Ignition temperature	363 °C (DIN 51794)	Solubility in water	(20 °C) soluble
Melting point	-114.5 °C	Molar mass	46.07 g/mol
Density	0.790-0.793 g/cm³ (20 °C)	pH	7.0 (10 g/l, H_2O, 20 °C)
Boiling point	78.3 °C	Vapor pressure	59 hPa (20 °C)
Explosive limits	3.5-15 % (V)	Flash point	13 °C c.c.
Refractive index	1.36		

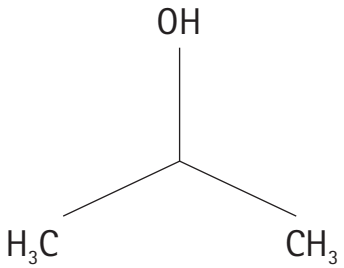
Ethanol is used in an undenatured grade with a purity of >/= 99.9 % or else as a 96 % solution. The undenatured grades [in Germany] have certain taxes imposed on them, making them quite pricey.
Besides the undenatured grade there is also denatured ethanol, which contains about 1 % MEK (methyl ethyl ketone). This denaturant has been used for many years and has proven so popular because it has no bearing on histological results. Being denatured, it does not attract special taxes so that this grade should be given preference in routine applications.
In addition to concentrated ethanol solutions procedures often call for diluted ones. These can be prepared manually as required.
For re- and de-hydrations 50 %, 70 % and 80 % solutions are used. Ethanol comes, of course, in numerous grades ranging from GR for analysis right down to technical grade, and also as reworked solutions. As elsewhere, the rule is that using good solvent grades tends to prevent problems in the work-flow and with the results. A realistic cost-benefit analysis should be conducted from time to time to enable changes to be made where necessary.

Ordering information

Name	Packaging	Ord. No.
Ethanol absolute, for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 l, 2.5 l	100983
Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 l, 5 l	100974

2-Propanol

2-Propanol, C_3H_8O, is an alcohol used as a laboratory solvent. 2-Propanol can also be used for the alcohol series of ascending concentrations for rehydrating and dehydrating specimens. It acts more slowly than ethanol.



Being flammable and irritant, 2-propanol bears the F and Xi hazard symbols.

2-Propanol is a solvent having the following physicochemical properties

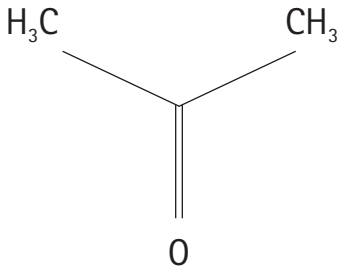
Chemical and physical data			
Ignition temperature	425 °C (DIN 51794)	Solubility in water	(20 °C) soluble
Melting point	-89.5 °C	Molar mass	60.1 g/mol
Density	0.786 g/cm³ (20 °C)	pH	(H_2O, 20 °C) neutral
Boiling point	82.4 °C (1013 hPa)	Vapor pressure	43 hPa (20 °C)
Explosive limits	2-13.4 % (V)	Flash point	17 °C, open crucible
Refractive index	1.378	Water absorption	1000 g/kg
Evaporation index	11		

Ordering information

Name	Packaging	Ord. No.
2-Propanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 l, 2.5 l	109634

Acetone

Acetone (synonyms dimethyl ketone, propanone), C_3H_6O, is a commonly used solvent that finds special applications in the histological laboratory: e.g. as a fixative and for addition to solutions.



Being flammable and irritant, acetone bears the F and Xi hazard symbols.

Acetone is a solvent having the following physicochemical properties

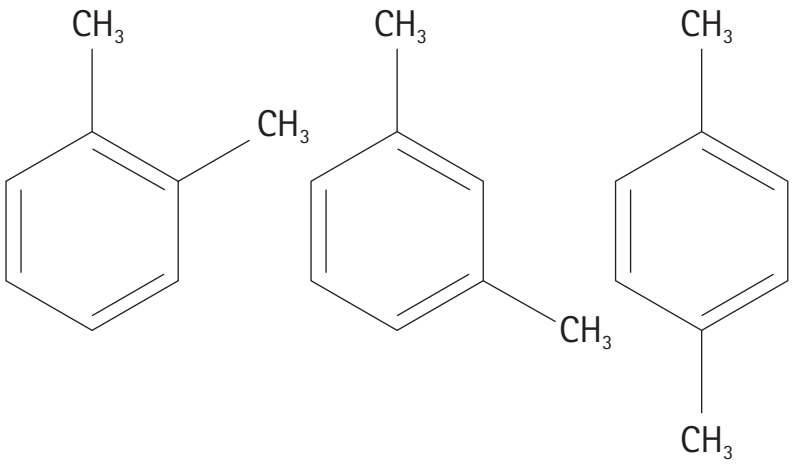
Chemical and physical data			
Ignition temperature	465 °C (DIN 51794)	Solubility in water	(20 °C) soluble
Melting point	-95.4 °C	Molar mass	58.07 g/mol
Density	0.79 g/cm³ (20 °C)	pH	5-6 (395 g/l, H_2O, 20 °C)
Boiling point	56.2 °C (1013 hPa)	Vapor pressure	233 hPa (20 °C)
Explosive limits	2.6-12.8 % (V)	Refractive index	1.35868 (20 °C)
Water absorption	1000 g/kg		

Ordering information

Name	Packaging	Ord. No.
Acetone for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 l, 2.5 l	100014

Xylene

Xylene is an aromatic solvent, $C_6H_4(CH_3)_2$, which has been used for decades in histoprocessing, deparaffination and clarification applications. A mixture of the ortho-, meta- and para-isomers of xylene is used.



Xylene is a solvent having the following physicochemical properties

Chemical and physical data			
Ignition temperature	490 °C (DIN 51794)	Solubility in water	0.2 g/l (20 °C)
Melting point	> -34 °C	Molar mass	106.17 g/mol
Density	0.86 g/cm³ (20 °C)	pH	(H_2O) not applicable
Boiling point	137-143 °C (1013 hPa)	Vapor pressure	10 hPa (20 °C)
Explosive limits	1.7-7.0 % (V)	Flash point	25 °C
Saturation concentration	35 g/m³ (20 °C) air	Kinematic viscosity	0.85 mm²/s (201 °C)
Evaporation index	13.5		

Xylene

The advantage of xylene is that it deparaffinizes sections very quickly and efficiently and also ends dehydration. Xylene has excellent capacity and speed for dissolving paraffin from the sections. In histology, these properties have made xylene the standard solvent.
Tried-and-tested protocols for using xylene in histology can be found in the Histoprocessing and Staining sections.

Various grades of xylene are available ranging from GR for analysis to technical grade, but difficulties may be encountered when the less good grades are used. The stain and also its durability may be adversely affected when the xylene is of a grade that is too low.
Xylene is a solvent that can be reworked. There are systems on the market that allow spent xylene to be processed, i.e. distilled, so it can be recycled and re-used. It should be bearing in mind, however, that this type of reworking is always associated with a certain loss of quality and any xylene that has been recycled must be replaced before quality loss in the specimen material being treated can occur.

The Problem

The problem with xylene, a hazardous substance with an Xn classification, is that it is flammable, harmful and an irritant, and has a high evaporation rate and a characteristic odor. The odor quickly reveals where xylene is being used. Laboratories must have good ventilation and any work involving xylene must be carried out under an extraction hood.
Safety instructions and other information on how to handle xylene without risk are contained in the safety data sheet which is available via the Web or upon request.

Aromatic solvents such as xylene are known to cause damage to the liver, hematopoetic organs and the lymphatic system as a result of intense exposure. The LD 50 oral dose for the rat is 2840 mg/kg; LD 50 dermal for the rabbit is > 4350 mg/kg.

Ordering information

Name	Packaging	Ord. No.
Xylene for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 l, 2.5 l	108661

Neo-Clear®

Neo-Clear® substitute for xylene

To meet the increasingly stringent regulations imposed by the health and environmental authorities, there is a need for aromatic solvents such as xylene to be replaced with user- and eco-friendly non-aromatic solvents.

Required features of a xylene substitute

- Reduced health risk
- Identical results
- Few, if any, changes to work instructions
- Same useful life
- Little, preferably no, environmental impact

In practice, a xylene substitute is used that originates from different chemical groups: from the terpenes, with their citrus-like odor, and from the group of aliphatic hydrocarbon mixtures having a chain length of about C_7-C_{15}, which places them among the isoparaffins.

Neo-Clear® is a saturated aliphatic C_9-C_{11} hydrocarbon mixture, CAS No. 64742-48-9/265-150-3/649-327-00-6, which is virtually odorless, colorless, miscible with most organic solvents, immiscible with water.

Optimum results with Neo-Clear® – technical information

The normal protocol for xylene can be used for histoprocessing. Application testing has shown xylene and Neo-Clear® to have the same lifetimes in the histoprocessor.

There are no differences in the way they are used in deparaffination. Neo-Clear® should be replaced in the same rhythm as xylene.

No clouding of alcohol baths in the staining series is observed.

Clarification with Neo-Clear® delivers color-fast slides in the same quality as when xylene is used.

To achieve optimum staining results, the specimens must be mounted using a suitable mounting medium. We sell Neo-Mount™, Ord. No. 109016, for coverslipping – a special mounting medium that is designed for use with Neo-Clear®.

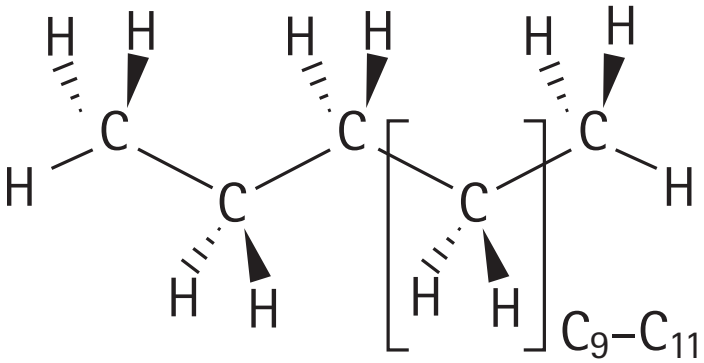
Neo-Clear® is an aliphatic hydrocarbon (C_9-C_{11}) belonging to the group of substances known as isoparaffins. These solvents are able to dissolve paraffin, a crucial feature in a xylene substitute, and have a slightly oily consistency which means they react very sensitively when they come into direct contact with water.

One or two points are mentioned below and that will help users gain the most from Neo-Clear® and maximize the quality of their slides.

According to EC regulations, Neo-Clear® is classified as an Xn (harmful) substance. Safety instructions and other information on how to handle Neo-Clear® without risk are contained in the safety data sheet which is available via the Web or upon request.

Neo-Clear® is a saturated aliphatic C_9–C_{11} hydrocarbon mixture

Chemical and physical data			
Ignition temperature	~ 230 °C (DIN 51794)	Solubility in water	0.1 g/l (20 °C)
Density	0.77 g/cm³ (20 °C)	Vapor pressure	7.1 hPa (20 °C)
Boiling point	150–215 °C (1013 hPA)	Flash point	> 40 °C
Explosive limits	0.7–7.0 % (V)		



Neo-Clear® is an aliphatic hydrocarbon mixture with a chain length of C_9-C_{11}

Neo-Clear® and Neo-Mount™

The perfect product combination for histoprocessing and mounting

Neo-Clear® and alcohol quality

Turbidity does not occur in the series of alcohol baths when "extra pure" ethanol or better is used in a concentration of 96 % or more. Technical grades of alcohol may have a lower ethanol content than stated and may not be concentrated enough to achieve full dehydration, with the consequence that water is carried over to the Neo-Clear® baths, causing the medium to become turbid and giving coverslipped slides a cloudy appearance.

Neo-Clear® and manual mounting with Neo-Mount™

Neo-Clear® has a lower rate of evaporation than xylene, necessitating minor changes to the normal protocol.
Once the slides have been removed from the final bath in the dehydration/clarification series, they are normally processed briskly from then on to stop them drying out.
Specimens treated with Neo-Clear® should be mounted with the matching mounting medium, Neo-Mount™, in order to ensure that the usual optical brilliance is achieved. Xylene-based mounting media cause smears and cloudiness in slides prepared using Neo-Clear®.
When Neo-Clear® is used for clarification and Neo-Mount™ for mounting, the excess Neo-Clear® must be removed from the specimens by placing them on a sheet of filter paper for approx. 1 minute. The risk of specimens drying out does not exist owing to the lower rate of evaporation of Neo-Clear® and to its slightly oily consistency. The slides are subsequently coverslipped in the usual way.
If the excess Neo-Clear® remains on the specimens and these are then mounted with Neo-Mount™ and coverslipped, air bubbles may form underneath the coverslip.

Neo-Clear® and mounting with Neo-Mount™ using an automatic coverslipper

When specimens that have been clarified with Neo-Clear® are covered using an automatic coverslipper, the specimens in the holding vessel prior to coverslipping must not be covered with Neo-Clear® (which, by contrast, is essential when xylene is used), but must instead be placed in the empty holding vessel. The specimens do not dry out, even when the rack is completely full of slides. If the specimens are directly coverslipped from a container filled with Neo-Clear®, it can happen that all of the mounted specimens will be completely covered with air bubbles underneath the coverslip within just a few hours.

Use of Neo-Clear® and Neo-Mount™ produces color-fast slides. The drying time for slides coverslipped using Neo-Mount™ is about 30 minutes.

If necessary, coverslips can be removed again by immersing them in Neo-Clear®.

Ordering information

Name	Packaging	Ord. No.
Neo-Clear®	5 l, 25 l	109843
Neo-Clear®	4 x 5 l	1.09843.5004

Suitable Mounting medium

Neo-Mount™	100 ml, 500 ml	109016

Stains

Ready-to-use staining solutions for histology

Your benefits

- Ready to use
- Proven staining protocols
- Intensive staining results
- Reproducible results
- High yields
- Batch-to-batch constancy
- Stable for at least 2, mostly 3 years
- Safe to use
- Quality controls and application testing
- No direct contact with powder dyes or dust

Overview and standard staining with hematoxylin and eosin
H&E staining is a standard histological procedure, yielding a good overview of tissue structures and at the same time enabling structures of interest to be categorized as normal, inflamed, degeneratively changed, or pathological.

In the H&E stain the nuclei are first stained with an acidic hematoxylin solution in which the hematoxylin is in oxidized form as hematein. In addition to the dye the solution contains a trivalent metal salt, here an aluminum compound, and an oxidizing agent, now an iodate instead of the mercury salts previously used. In the solution the dye forms a complex with the metal salt, so staining the target structures. To allow the complex hematoxylin compound to assume the typical blue color, the slide is rinsed in the next step in running tap water, which fixes the stain on the target structures. The nuclei stain dark blue, dark violet to black.

As well as the nuclei, staining turns calcified tissue, acidic mucus and gram-positive bacteria blue, too.

Nuclear staining with hematoxylin

In hematoxylin-eosin staining, the most widely used histological staining technique, nuclear staining is achieved using ready-to-use staining solutions: Mayer's hemalum solution or Hematoxylin solution modified according to Gill III. Counterstaining is performed with aqueous Eosin Y solution 0.5 %, alcoholic Eosin Y solution 0.5 % and 1 % or alcoholoc Eosin-Phloxin solution, i.e. for staining proteine, collagen, keratin, intercellular substances.

Both hematoxylin solutions use aluminum salts for the complexing reaction. These belong to the group of so-called hemalums and behave in a similar manner in terms of staining times, differentiation and finished stain.

The benefits provided by ready-to-use hematoxylin solutions for histology as compared to self-prepared solutions, in addition to the features already mentioned, are as follows ...

Nuclear staining with hematoxylin

- Controlled oxidation of the dye in the solution
- Stable results with selective nuclear staining
- Choice of classical or modified solution

H&E staining

Counterstaining with eosin

Following nuclear staining, counterstaining must be performed to visualize proteins, connective tissues, fibers and keratin.

The dye most widely used for this purpose is Eosin Y, where Y stands for yellowish. Depending on requirements and personal preferences most laboratories use concentrations of between 0.1 % and 1 % eosin in aqueous or alcoholic staining solutions.

Eosin Y solution 0.5 % alcoholic, Eosin Y solution 1 % alcoholoc and Eosin Y solution 0.5 % aqueous are available for H&E counterstaining in the form of ready-to-use staining solutions.

Beside the pure Eosin Y solution is available an alcoholic Eosin-Phloxin solution for a more intense stain because the combination of both dyes results in a broader color range in the tissue.

Addition of acetic acid to either one of these solutions produces a more brilliant stain (add approx. 0.2 ml of acetic acid (glacial) 100 % anhydrous to 100 ml of Eosin Y solution).

All alcoholoic and aqueous Eosin Y solution and the Eosin-Phloxin solution can be used with Mayer's hemalum solution or with hematoxylin solution modified according to Gill III.

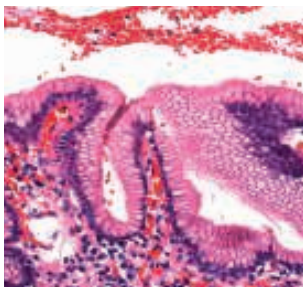
Protocol H&E staining – Eosin Y **aqueous**

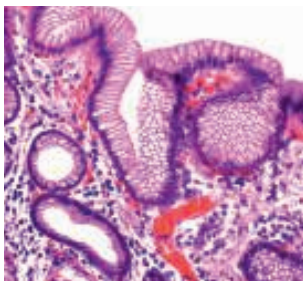
Step	Time
Deparaffinize the sections in the typical manner and rehydrate	
Stain in Mayer's hemalum solution, or Hematoxylin solution acc. to Gill III	3 min
Rinse in 0.1 % HCl solution	2 sec
Differentiate in running tap water	3-5 min
Eosin Y solution 0.5 % **aqueous***	3 min
Rinse in tap water	30 sec
Ethanol 70 %	20 sec
Ethanol 70 %	20 sec
Ethanol 96 %	20 sec
Ethanol 96 %	20 sec
Ethanol 100 %, denatured	20 sec
Ethanol 100 %, denatured	20 sec
Clear in Neo-Clear® or xylene	5 min
Clear in Neo-Clear® or xylene	5 min
Mount the Neo-Clear®-wet slides with Neo-Mount™ and the xylene-wet slides with Entellan™ new and cover glass	

*Add 0.2 ml Glacial acetic acid to 100 ml eosin Y solution 0,5 % aqueous for more intense staining result

Material

As well as for paraffin sections and frozen tissue sections, the H&E stain can be used for cytological material such as sputum, lavages, urine sediments, effusions and FNAB specimens.





Intestine, paraffin section, H&E stain

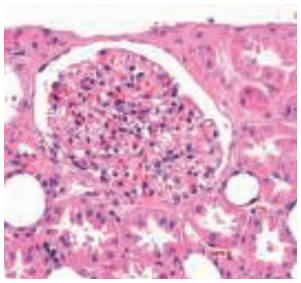
Protocol H&E staining – **alcoholic** Eosin Y or Eosin-Phloxin

Step	Time
Deparaffinize the sections in the typical manner and rehydrate	
Stain in Mayer's hemalum solution, or Hematoxylin solution acc. to Gill III	3 min
Rinse in 0.1 % HCl solution	2 sec
Differentiate in running tap water	3-5 min
Eosin Y solution 0.5 % or 1 % **alcoholic** or Eosin-Phloxin solution*	3 min
Ethanol 96 %	10 sec
Ethanol 96 %	10 sec
Ethanol 100 %, denatured	10 sec
Ethanol 100 %, denatured	10 sec
Clear in Neo-Clear® or xylene	5 min
Clear in Neo-Clear® or xylene	5 min
Mount the Neo-Clear®-wet slides with Neo-Mount™ and the xylene-wet slides with Entellan™ new and cover glass	

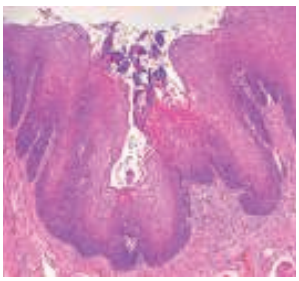
*Add 0.2 ml Glacial acetic acid to 100 ml eosin Y solution 0,5 % alcoholic for more intense staining result

Result	
Nuclei	dark blue to dark violet
Cytoplasm, intercellular substances	red-orange
Erythrocytes	yellow-orange

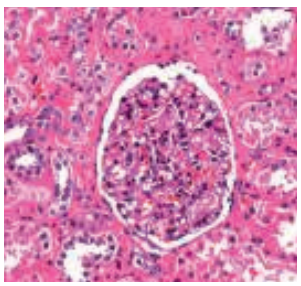
When specimens of frozen tissue sections are to be visualized using an H&E stain, it is important that the unfixed material reveals all of its structural details in a clarity that permits easy assessment. As the dye binds much more quickly to the target structures of unfixed material, staining times must be significantly reduced (see protocol for frozen tissue specimens). Neo-Clear® and Neo-Mount™ bring numerous benefits in comparison with xylene and matching mounting medium, as the product combination is virtually odorless and almost evaporation-free.



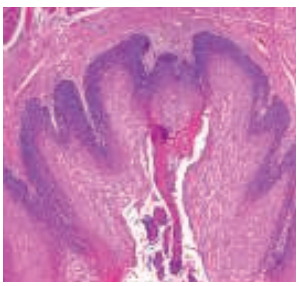
Kidney, paraffin section, H&E stain, Eosin Y 0.5% aqueous



Tongue, H&E stain, Eosin Y 1% alcoholic



Kidney, paraffin section, H&E Eosin Y 0.5% alcoholic



Tongue, paraffin section, H&E stain, Eosin-Phloxin alcoholic

If different dye concentrations are needed in the hematoxylin and eosin solutions, these can be prepared using Certistain® grade solid dyes.

Protocol for cryo sections

Step	Concentration	Time
Nuclear staining with hematoxylin solution acc. to Gill III		10-20 sec
Rinse in running tap water		Move sections until no further red clouds leave the normally thick parts of the preparations
Differentiate in acetic acid	1 %	10 sec
Differentiate in tap water		Move sections, until the color changes from violet to blue
Rinse in distilled water		Rinse shortly
Stain in Eosin Y solution alkoholic	0.5 %	10-15 sec
Stain in Eosin Y solution alkoholic	1 %	10 sec
Stain in Eosin-Phloxin solution alcoholic		10 sec
Dehydrate in Ethanol	70 %	Move sections until no further color clouds leave the preparations
Dehydrate in Ethanol	96 %	Move sections until no further color clouds leave the preparations
Dehydrate in Ethanol	100 %	Move sections until no further color clouds leave the preparations
Clear in Neo-Clear®		15 sec
Mount with Neo-Mount™		

Result	
Nuclei	dark blue to dark violet, black
Proteine, connective tissue, collagen, keratin	red-orange, yellow-orange

Ordering information

Name	Packaging	Ord. No.
Mayer's Hemalum solution	500 ml, 1 l, 2,5 l	109249
Hematoxylin solution modified acc. to Gill III	500 ml, 1 l, 2,5 l	105174
Eosin Y solution alcoholic 0,5 %	500 ml, 2,5 l	102439
Eosin Y solution alcoholic 1 %	1 l	117081
Eosin-Phloxin solution alcoholic	500 ml, 1 l	102480
Eosin Y solution aqueous 0,5 %	1 l, 2,5 l	109844

PAS staining

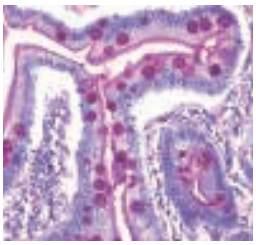
PAS (periodic acid-Schiff) staining is one of the most widely used chemical methods in histology.
In PAS staining the material is treated with periodic acid during which process the 1,2-glycols are oxidized to aldehyde groups. With Schiff's reagent aldehydes produce a bright red color. With unsubstituted polysaccharides, neutral mucopolysaccharides, mucoproteins, glycoproteins, glycolipids and phospholipids, PAS staining produces a specific color reaction.
Combining PAS staining with Alcian blue, at a pH of 2.5 for the Alcian blue solution, allows the additional identification of acid mucosubstances (glycosaminoglycans). At pH 1 the sulfated mucosubstances are stained. PAS staining is used for paraffin sections and smears.
To further enhance the brillliance and contrast of the PAS-positive structures, it is recommended to use hematoxylin solution modifi ed according to Gill II (Cat. No. 105175).

Schiff's reagent for the periodic acid Schiff, or PAS, reaction – for detection of mucopolysaccharides, glycogen, neutral mucopolysaccharides, muco- and glycoproteins, glycolipids, phospholipids, basement membrane, collagen.

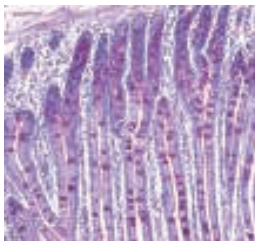
Protocol for PAS staining

Step	Time
Deparaffinize the sections in the typical manner and rehydrate	
Rinse in tap water	5 min
Periodate solution	5 min
Rinse in distilled water	
Schiff's reagent	15 min
Rinse in sulfite water	3 x 2 min
Rinse under running tap water	10 min
Hematoxylin solution acc. to Gill III or Gill II	2 min
Running tap water	3 min
Ascending alcohol series, 2 x Neo-Clear® or xylene	
Mount the xylene-wet slides with Entellan™ new or the Neo-Clear®-wet slides with Neo-Mount™ and cover glass	

Result	
Nuclei	blue
Polysaccharides, glycogen, neutral mucopolysaccharides, muco- and glycoproteins, glycolipids, phospholipids, basal membrane, collagen	purple



Duodenum, paraffin section, PAS stain



Duodenum, paraffin section, PAS stain

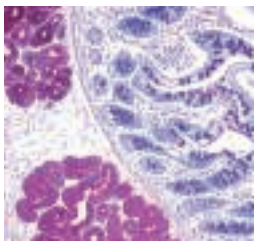
Ordering information

Name	Packaging	Ord. No.
Schiff's reagent	500 ml, 2.5 l	109033

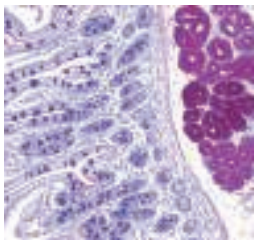
Additional required

Name	Packaging	Ord. No.
Hematoxylin solution modified acc. to Gill III	500 ml, 1 l, 2.5 l	105174
Hematoxylin solution modified acc. to Gill II	500 ml, 2.5 l	105175

Alcian blue-PAS



Duodenum, paraffin section, Alcian blue-PAS



Duodenum, paraffin section, Alcian blue-PAS

Acid mucosubstances and polysaccharides and neutral mucopolysaccharides are visualized using a combination of Alcian blue (pH 2.5) and the PAS stain.

Protocol for Alcian blue-PAS staining

Step	Time
Deparaffinize the sections in the typical manner and rehydrate	
Alcian blue solution	5 min
Tap water, running	3 min
Rinse in distilled water	
Periodic acid	10 min
Tap water, running	3 min
Rinse in distilled water	
Schiff's reagent	15 min
Tap water, running	3 min
Rinse in distilled water	
Hematoxylin solution acc. to Gill III	20 sec
Tap water, running	3 min
Ascending alcohol series, 2 x Neo-Clear® or xylene	
Mount the xylene-wet slides with Entellan™ new or the Neo-Clear®-wet slides with Neo-Mount™ and cover glass	

Result	
Nuclei	blue
Acid mucosubstances	brightly, light-blue
Polysaccharides, neutral mucopolysaccharides	purple

Ordering information

Name	Packaging	Ord. No.
Schiff's reagent	500 ml, 2.5 l	109033
Alcian blue solution	500 ml	101647

Additional required

Name	Packaging	Ord. No.
Hematoxylin solution modified acc. to Gill III	500 ml, 1 l, 2.5 l	105174

Nuclear fast red staining

Nuclear fast red-aluminum sulfate solution 0.1 % for nuclear staining and overview staining
Nuclear fast red staining is a simple and reliable method of nuclear staining and is mainly used for high-contrast counterstaining. An aluminum lake of nuclear fast red (nuclear fast red-aluminum sulfate) actually produces the nuclear stain. Background and cytoplasm staining is achieved through free dye that is not bound in the solution as aluminum dye lake. Nuclear fast red solution for nuclear staining is used for example in the Alcian blue stain, for visualization of free iron with the Prussian blue reaction (see staining kits: HEMATOGNOST Fe™) and in the silver stain for visualization of reticular fibers (see Reticulin silver plating kit acc. to Gordon & Sweets).
If only cytoplasm is to be stained, an acidified aqueous 0.1 % nuclear fast red solution not containing aluminum sulfate is used.

Nuclear staining overview staining with nuclear fast red
Visualization of nuclei and all cell structures with nuclear fast red/aluminum sulfate solution 0.1 %.

Protocol for Nuclear staining/Overview staining

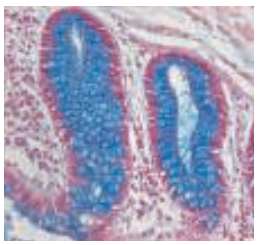
Step	Concentration	Time
Deparaffinize the sections in the typical manner and rehydrate		
Rinse in distilled water		1 min
Nuclear fast red-aluminum-sulfate solution 0.1 %		10 min
Rinse in distilled water		1 min
Ethanol	70 %	1 min
Ethanol	96 %	1 min
Ethanol	100 %	1 min
Ethanol	100 %	1 min
Xylene or Neo-Clear®		5 min
Xylene or Neo-Clear®		5 min
Mount the xylene-wet slides with Entellan™ new and the Neo-Clear®-wet slides with Neo-Mount™		

Result	
Nuclei	dark red
Cytoplasm	light red

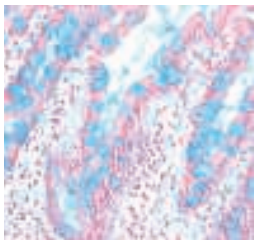
Ordering information

Name	Packaging	Ord. No.
Nuclear fast red-aluminum sulfate solution 0.1 %	500 ml	100121

Alcian blue-nuclear fast red



Intestine, paraffin section, Alcian blue-nuclear fast red-aluminum sulfate 0.1 % solution



Stomach, paraffin section, Alcian blue-nuclear fast red-aluminum sulfate 0.1 % solution

If only acid mucosubstances are to be stained, use of the Alcian blue stain (pH 2.5) is recommended; with nuclear fast red counterstaining for better structural vizualization of the nuclei.

Protocol for Alcian blue staining for acid mucosubstances

Step	Concentration	Time
Deparaffinize the sections in the typical manner and rehydrate		
Alcian blue solution		5 min
Running tap water		3 min
Rinse in distilled water		
Nuclear fast red-aluminum sulfate solution 0.1 %		10 min
Running tap water		3 min
Rinse in distilled water		
Ethanol	70 %	1 min
Ethanol	96 %	1 min
Ethanol	100 %	1 min
Ethanol	100 %	1 min
Xylene or Neo-Clear®		5 min
Xylene or Neo-Clear®		5 min
Mount the xylene-wet slides with Entellan™ new and the Neo-Clear®-wet slides with Neo-Mount™		

Result	
Elastic fibers	black
Nuclei	red
Cytoplasm	light red

Ordering information

Name	Packaging	Ord. No.
Alcian blue solution	500 ml	101647
Nuclear fast red-aluminum sulfate solution 0.1 %	500 ml	100121

Van Gieson's staining

Picrofuchsin solution for van Gieson's trichrome staining

The van Gieson's stain is a frequently used means of achieving high-contrast visualization of collagenous connective tissue in paraffin sections. Van Gieson's picrofuchsin solution is used together with Weigert's iron hematoxylin solution, Ord. No. 115973, in a trichrome stain. Van Gieson's picrofuchsin solution contains two differently dispersed dyes: finely dispersed picric acid and coarsely dispersed acid fuchsin. The stain turns collagenous fibers bright red, and muscles, cytoplasm and glial fibers yellow. Amyloid, hyaline, colloid and mucus exhibit a gradated stain that ranges from yellow to red tones. For stable, durable nuclear staining Weigert's iron hematoxylin should be used so as to avoid fading of the stain through the acidic staining solution.

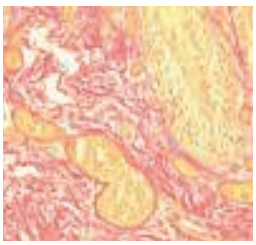
The advantage of using the ready-to-use van Gieson's picrofuchsin solution lies in the fact that the picric acid is in dissolved form and direct contact with it can be avoided, as can also direct contact with acid fuchsin dye. Van Gieson's picrofuchsin solution can also be used in combination with Weigert's elastin solution in the Elastica van Gieson's stain to demonstrate elastic fibers in histological material.

Material

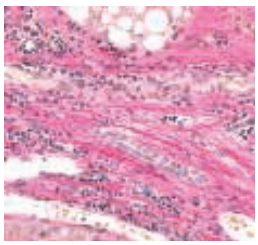
3 µm thick paraffin sections, fixed in formalin or Bouin's solution.

Preparation

Weigert's iron hematoxylin solution
Mix Weigert's solution A and Weigert's solution B in the ratio 1:1.



Uterus, paraffin section, Picrofuchsin solution



Tongue, paraffin section, Picrofuchsin solution

Protocol for Trichrome staining acc. to van Gieson

Step	Concentration	Time
Deparaffinize the sections in the typical manner and rehydrate		
Weigert's iron hematoxylin solution		5 min
Rinse in running tap water		3 min
Picrofuchsin solution		30 sec
Rinse in distilled water		30 sec
Ethanol	96 %	30 sec
Ethanol	96 %	30 sec
Ethanol	100 %	1 min
Ethanol	100 %	1 min
Xylene or Neo-Clear®		5 min
Xylene or Neo-Clear®		5 min
Mount the xylene-wet slides with Entellan™ new and the Neo-Clear®-wet slides with Neo- Mount™		

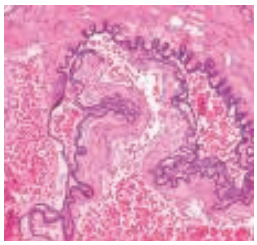
Result	
Nuclei	black-brown
Collagen	red
Muscle tissue, glia fibrils	yellow
Colloid, mucus, hyalin, amyloid, cornified epithelium	yellow to red

Ordering information

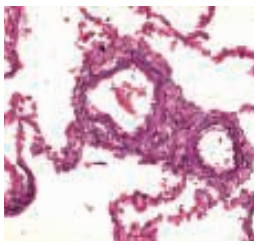
Name	Packaging	Ord. No.
Picrofuchsin solution acc. to van Gieson	500 ml	100199
Weigert's iron hematoxylin kit	2 x 500 ml	1.15973.0002

Elastin staining

Elastin solution acc. to Weigert is used for visualisation of elastic fibers stain in paraffin sections. Elastic fibers are bundles of microfibrils (glycoprotein) that accumulate elastin in their development (polymeric protein), gradually resulting in the formation of coarse elastic fibers and membranes. The microfibrils on the surface of the elastic fibers expose high numbers of disulfide groups, which can be chemically detected when sufficient material is available for light-microscopic investigations. Furthermore, they are strongly acidic and thus bind the resorcin-fuchsin dye by electropolarity via interface adsorption. The resorcin-fuchsin staining method is a regressive method. In tissue sections, nuclei can be stained using a 0.1 % nuclear fast red-aluminum sulfate solution if not only elastic fibers, but also nuclei and the background also need to be visualized.



Duodenum, paraffin section, Elastin-Nuclear fast red solution



Lung, Paraffin section, Elastin-Nuclear fast red solution

Protocol for Elastin staining

Step	Time
Deparaffinize the sections in the typical manner and rehydrate	
Elastin solution acc. to Weigert	15 min
Running tap water	1 min
Nuclear fast red-aluminum sulfate solution 0.1 %	5 min
Running tap water	1 min
Rinse with distilled water	5–10 sec
Ethanol 70 %	1 min
Ethanol 70 %	1 min
Ethanol 96 %	1 min
Ethanol 96 %	1 min
Ethanol 100 %	1 min
Ethanol 100 %	1 min
Clarify with Neo-Clear® or xylene	5 min
Clarify with Neo-Clear® or xylene	5 min
Mount the Neo-Clear®-wet slides with Neo-Mount™ or the xylene-wet slides with Entellan® new and cover glass	

Result	
Elastic fibers	black
Nuclei	red
Cytoplasm	light red

Ordering information

Name	Packaging	Ord. No.
Elastin solution acc. to Weigert	500 ml	100591
Nuclear fast red-aluminium sulfate solution 0.1 %	500 ml	100121

Oil red O staining

The staining of triglycerides in histology specimens is a physical process.
Oil red O is a lipophilic dye; due to its solubility characteristics it diffuses to the tissue lipids.
The stain is carried out on cryo sections of native tissue.

Material

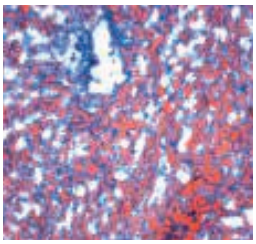
Cryo sections 8–10 µm

Oil red O color solution for the detection of neutral lipids in cryo sections for microscopy

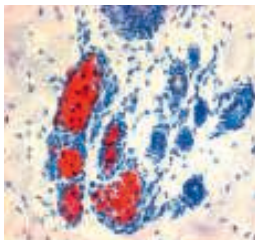
Protocol for Oil red O staining

Step	Time
Slide with cryo section	
2-Propanol 60 %	20 sec
Oil red O color solution	10 min
2-Propanol 60 %	30 sec
Distilled water	20 sec
Hematoxylin solution modified acc. to Gill II	5 min
Running tap water	3 min
Mount the wet slides with an aqueous mounting medium (e. g. Aquatex®) and cover glass.	

Result	
Nuclei	blue
Triglycerides	bright red



Liver, cryo section, Oil red O, Hematoxylin acc. to Gill II



Mammary gland, cryo section, Oil red O, Hematoxylin acc. to Gill II

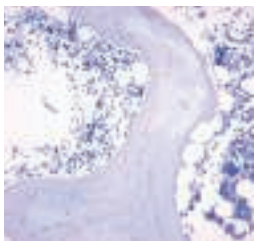
Ordering information

Name	Packaging	Ord. No.
Oil red O color solution	250 ml	102419

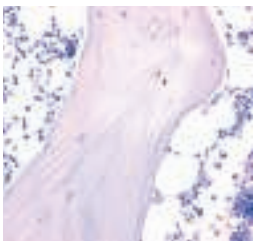
Also required

Name	Packaging	Ord. No.
Hematoxylin solution modified acc. to Gill II	500 ml, 2.5 l	105175

Giemsa's staining



Bone marrow biopsy, paraffin section, Giemsa's azure eosin methylene blue solution



Bone marrow biopsy, paraffin section, Giemsa's azure eosin methylene blue solution

Azure eosin methylene blue solution according to Giemsa's
Giemsa's staining is one of the standard procedures in histology, used to stain special materials such as bone marrow, tonsils and lymph nodes on account of their high proportion of hematologie and lymphatic cells. Staining demonstrates the various cells with their morphological features better than can be achieved with an H&E stain. The Giemsa's stain can also be used to detect *Helicobacter pylori* in gastric tissue biopsies. The specimen material is affected by pre-treatments such as fixation and histoprocessing; cell nuclei are stained various shades of blue, while other structures are visualized through various red tones.

For Giemsa's staining of paraffin sections it is important to use separate clarification baths with xylene or Neo-Clear®, as traces of ethanol in the solutions can cause slides to become decolored. Pre-treatment of bone marrow and punched iliac crests: Optimum results are achieved using OSTEOSOFT® mild decalcification solution. The fixed punch specimens are placed for 6 hours in OSTEOSOFT® for gentle decalcification, and afterwards undergo histoprocessing. Blocks are carefully sliced and, if necessary, post-treated for 20 min with OSTEOSOFT®.

Protocol Giemsa's staining

Step	Time
Deparaffinize the sections in the typical manner and rehydrate	
Rinse in distilled water	10 sec
Stain in Giemsa's azure eosin methylene blue solution undiluted, filtrated	15 min
Acetic acid 0.1 %	10 sec
Rinse in distilled water	10 sec
2-Propanol	10 sec
2-Propanol	10 sec
2-Propanol	10 sec
Xylene or Neo-Clear®	5 min
Xylene or Neo-Clear®	5 min
Mount the xylene-wet slides with Entellan™ new and the Neo-Clear®-wet slides with Neo-Mount™	

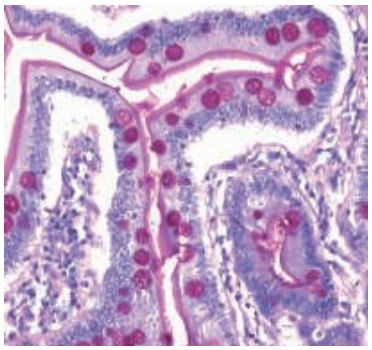
Result	
Cell nuclei, cells	blue, dark blue
Collagen, osteoid	pale blue
Eosinophilic grains	red
Acidophilic mucopolysaccharides, mastocytes, cartilage matrix	reddish violet
Acidophilic materials	orange red

Ordering information

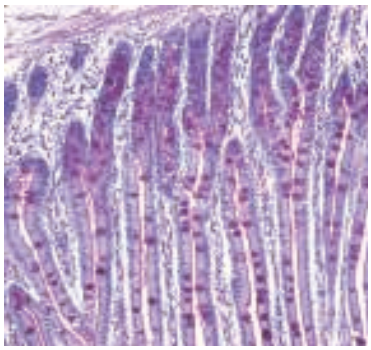
Name	Packaging	Ord. No.
Giema's azure eosin methylene blue solution	100 ml, 500 ml, 1 l, 2.5 l	109204

Staining kits

Depending on the aim and material, a number of special staining techniques are routinely used in histopathology besides H&E staining. The methods are well known and have stood the test of time; with results that are mostly comparable, even with modifications of the method. The solutions are often traditionally user-prepared. The range of ready-to-use staining solutions and staining kits is growing. A high degree of reliability is achieved through standardized manufacturing processes, carefully matched starting materials and quality controls. The use of ready-to-use solutions/kits should be given consideration as a possible alternative to traditional methods. For frequently used stains we supply kits which guarantee high batch-to-batch consistency, reproducible results, long shelf-lives, also during use, and simple, safe handling. Tried-and-tested instructions are enclosed with each product.



Duodenum, paraffin section
PAS staining kit



Duodenum, paraffin section
PAS staining kit

PAS staining

Kit for detection of polysaccharides, glycogen, neutral mucopolysaccharides, muco- und glycolipids, collagen, basement membranes

For PAS staining the material is treated with periodic acid during which process the 1,2-glycols are oxidized to aldehyde groups. With Schiff's reagent aldehydes give a bright red color reaction.
With non-substituted polysaccharides, neutral mucopolysaccharides, mucoproteins and glycoproteins, glycolipids and phospholipids the PAS stain produces a specific color reaction.
To further enhance the brillliance and contrast of the PAS-positive structures, it is recommended to use hematoxylin solution modified according to Gill II (Cat. No. 105175).

Protocol PAS staining

Step	Time
Deparaffinize the sections in the typical manner and rehydrate	
Rinse in distilled water	
Periodic acid	5 min
Tap water, running	3 min
Rinse in distilled water	
Schiff's reagent	15 min
Tap water, running	3 min
Rinse in distilled water	
Hematoxylin solution acc. to Gill III or Gill II	2 min
Tap water, running	3 min
Ascending alcohol series, 2 x Neo-Clear® or xylene	
Mount the xylene–wet slides with Entellan™ new or the Neo-Clear®-wet slides with Neo-Mount™ and cover glass.	

Result	
Nuclei	blue
Polysaccharides, glycogen, neutral mucopolysaccharides, muco- and glycoproteins, glycolipids, phospholipids, basal membrane, collagen	purple

Ordering information

Name	Packaging	Ord. No.
PAS staining kit	2 x 500 ml	1.01646.0001
Kit components		
Solution 1: Periodic acid 0.5 %, aqueous	500 ml	
Solution 2: Schiff's reagent	500 ml	

Additional required

Name	Packaging	Ord. No.
Hematoxylin solution modified acc. to Gill III	500 ml, 1 l, 2.5 l	105174
Hematoxylin solution modified acc. to Gill II	500 ml, 2.5 l	105175

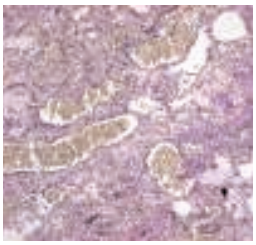
Weigert's iron hematoxylin

Kit for nuclear stains for trichrome staining and staining with acidic counterstains

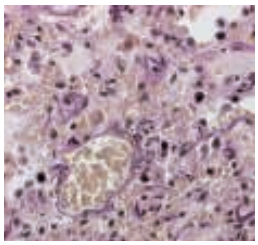
Iron hematoxylin solutions are used in particular for nuclear staining of tissue sections where counterstaining is performed using acidic dye solutions. Examples of this type of application are the demonstration of elastic fibers according to van Gieson's and the various trichrome connective tissue stains, due to the higher resistance of the iron hematoxylin stain to acidic staining solutions. Commonly used hemalum solutions such as Mayer's hemalum, Gill's and Harris' give only weak nuclear stains with acidic counterstaining.

Preparation

Weigert's iron hematoxylin solution
Mix Weigert's solution A and Weigert's solution B in the ratio 1:1.



Lung, paraffin section, Weigert's iron hematoxylin kit



Lung, paraffin section, Weigert's iron hematoxylin kit

Protocol Weigert´s iron hematoxylin staining procedure

Step	Time
Deparaffinize the sections in the typical manner and rehydrate	
Stain in Weigert's iron hematoxylin solution	5 min
Differentiate under running tap water	3 min
Counterstain according to the method being used	
Dehydrate, clarify and mount the sections	
Mount the xylene–wet slides with Entellan™ new or the Neo-Clear®-wet slides with Neo-Mount™ and cover glass	

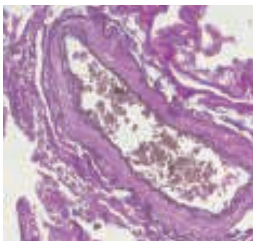
Result	
Nuclei	blue-black

Ordering information

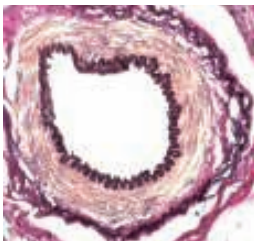
Name	Packaging	Ord. No.
Weigert's iron hematoxylin kit	2 x 500 ml	1.15973.0002
Kit components		
Weigert's solution A – Alcoholic hematoxylin solution	500 ml	
Weigert's solution B – Hydrochloric acid iron(III)nitrate solution	500 ml	

Elastica van Gieson's

Kit for detection of elastic fibers



Blood vessel, paraffin section, Elastica van Gieson's kit



Blood vessel, paraffin section, Elastica van Gieson's kit

Several common staining methods are used to demonstrate elastic fibers in tissue sections. Brilliant results are achieved for the visualization of fibers using resorcin-fuchsin solution according to Weigert's in association with van Gieson's picrofuchsin solution and nuclear staining with Weigert's iron hematoxylin solution.

Protocol for Elastica van Gieson's staining

Step	Time
Deparaffinize the sections in the typical manner and rehydrate	
Elastin according to Weigert's	10 min
Rinse under running tap water	1 min
Weigert's iron hematoxylin solution	5 min
Rinse under running tap water	3 min
Picrofuchsin solution	2 min
Ethanol 70 %	1 min
Increasing alcohol concentrations, 2 x xylene or Neo-Clear®	
Mount the xylene–wet slides with Entellan™ new or the Neo-Clear®-wet slides with Neo-Mount™ and cover glass	

Result	
Nuclei	black-brown
Elastic fibers	black
Collagen	red
Muscle	yellow

Ordering information

Name	Packaging	Ord. No.
Elastica van Gieson's kit	4 x 500 ml	1.15974.0002
Kit components		
Solution 1: Weigert's solution A – Alcoholic hematoxylin solution	500 ml	
Solution 2: Weigert's solution B – Hydrochloric acid iron(III)nitrate solution	500 ml	
Solution 3: Elastin acc. to Weigert's resorcin fuchsin solution	500 ml	
Solution 4: Picrofuchsin solution acc. to van Gieson (Picric acid/acid fuchsin solution)	500 ml	

Masson-Goldner

Kit for visualizing connective tissue with the Masson-Goldner trichrome stain

The combined use of three different staining solutions enables muscle fibers, collagen fibers, fibrin and erythrocytes to be selectively visualized. The original methods were primarily used to differentiate collagenous and muscle fibers. The stains used have different molecular sizes and enable the individual tissues to be stained differentially.

The Masson-Goldner stain has the following benefits: the technique can be applied using formalin-fixed material. Following nuclear staining with Weigert's iron hematoxylin, components such as muscle, cytoplasm and erythrocytes are stained with azophloxin and Orange G solution. Connective tissue is then counterstained with Light green SF solution.

Preparation

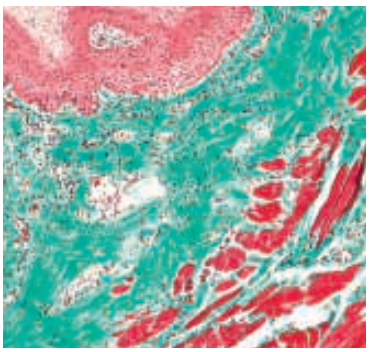
1. Acetic acid 1 %
   Dilute the 10 % acetic acid 10:1 with distilled water.

2. Weigert's iron hematoxylin solution
   Mix Weigert's solution A and Weigert's solution B in the ratio 1:1.

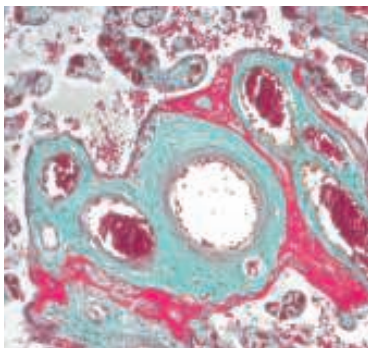
Protocol for Masson-Goldner trichrome staining

Step	Concentration	Time
Xylene or Neo-Clear®		5 min
Xylene or Neo-Clear®		5 min
Ethanol	100 %	30 sec
Ethanol	100 %	30 sec
Ethanol	96 %	30 sec
Ethanol	96 %	30 sec
Ethanol	70 %	30 sec
Ethanol		30 sec
Weigert's iron hematoxylin solution		5 min
Running tap water		5 min
Rinse in 1 % acetic acid		30 sec
Azophloxin solution		10 min
Rinse in 1 % acetic acid		30 sec
Tungstophosphoric acid Orange G solution		1 min
Rinse in 1 % acetic acid		30 sec
Light green SF solution		2 min
Rinse in 1 % acetic acid		30 sec
Ethanol	70 %	30 sec
Ethanol	96 %	30 sec
Ethanol	100 %	30 sec
Ethanol	100 %	30 sec
Ethanol	100 %	2 min
Xylene or Neo-Clear®		5 min
Xylene or Neo-Clear®		5 min
Mount the xylene–wet slides with Entellan™ new or the Neo-Clear®-wet slides with Neo-Mount™ and cover glass		
Acetic acid 1 %: Prepare fresh solution regularly. The times given should be adhered to if optimal results are to be obtained		

Result	
Nuclei	dark brown to black
Cytoplasm, muscle	brick red
Connective tissue, acidic mucus substances	green
Erythrocytes	bright orange



Tongue, paraffin section, Masson-Goldner Kit



Placenta, paraffin section, Masson-Goldner Kit

Ordering information

Name	Packaging	Ord. No.
Masson-Goldner staining kit	4 x 500 ml	1.00485.0001
Kit components		
Solution 1: Azophloxine solution	500 ml	
Solution 2: Tungstophosphoric acid Orange G solution	500 ml	
Solution 3: Light green SF solution	500 ml	
Solution 4: Acetic acid 10 %	500 ml	
Additional required		
Weigert's iron hematoxylin kit	2 x 500 ml	1.15973.0002

Methenamine silver plating acc. to Gomori (GMS stain)

Kit for visualizing basement membranes, enterochromaffin cells, bacteria and fungi

Silver staining

Silver staining is one of the commonest special staining techniques used in histology. Various reagents can be applied to visualize different tissue structures. Gomori's Methenamine Silver stain is used on basement membranes, bacteria and fungi. The target structures are turned black, while everything else is stained green with Light green SF solution.
Through GMS staining, aldehydes resulting from oxidation of 1,2-glycols with periodic acid are visualized with a methenamine borate silver nitrate solution. The aldehyde groups reduce silver ions in the alkaline pH range to metallic silver, which appears black. The color of the silver deposits can be intensified still further by treatment with gold chloride solution, through formation of a silver-gold complex. The use of gold chloride solution also prevents non-specific staining of the background. Excess silver nitrate is washed out with sodium thiosulfate solution.

A microwave protocol has been compiled for laboratories that use a microwave. The microwave reduces the time required for the silver nitrate/methenamine borate solution step to only 2 minutes following heating at 600 W for 55 seconds. The proven microwave protocol is available on request.

Preparation of the methenamine silver solution

1 tablet methenamine/borate is sufficient for the preparation of 30 ml of silver solution. Completely dissolve the tablet in silver solution. The solution is ready for use. Place the silver nitrate/methenamine borate solution together with the sample to be stained into the water bath previously heated to 55 °C, maintain this temperature throughout the staining process and stain for 35 to 45 minutes until achieving the desired intensity. Use the solution immediately and discard after use.

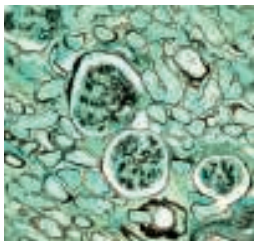
Important note

Use only clean glass and plastic vessels for the preparation of silver solution. Avoid contact of metal things (e.g. slide holder tweezers) with silver solution.

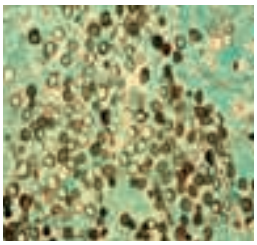
Protocol for Methenamine silver plating kit

Step	Time
Deparaffinize the sections in the typical manner and rehydrate	
Distilled water	2 min
Periodic acid solution	10 sec
Distilled water	3 x ca. 30 sec
Stain in the water bath at 52-57 °C with silver nitrate/methenamine borate solution	35-45 sec
Distilled water	3 x ca. 30 sec
Gold chloride solution	1 min
Distilled water	ca. 30 sec
Sodium thiosulfate solution	2 min
Running tap water	3 min
Distilled water	ca. 30 sec
Light green SF solution	2-3 min
Distilled water	ca. 30 sec
Dehydrate in ascending alcohol bath, 2 x clear in Neo-Clear® or xylene	
Mount the xylene–wet slides with Entellan™ new or the Neo-Clear®-wet slides with Neo-Mount™ and cover glass	

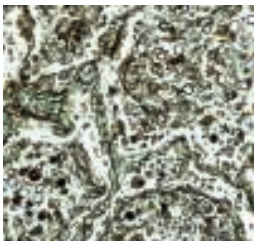
Result	
Fungi	dark brown to black
Basement membranes	dark brown to black
Background	green



Kidney, paraffin section, Methenamine silver plating kit acc. to Gomori



Lung with fungi, paraffin section Methenamine silver plating kit acc. to Gomori – with gold chlorided



Lung, with fungi paraffin section Methenamine silver plating kit acc. to Gomori – without gold chloride

Advantages of using gold chloride solution in silver plating reactions

The silver methods are sensitive reactions which can respond to disturbances in the procedure by producing an inadequate or nonspecific reaction. The working instructions should always be followed closely in order to achieve specific and distinct results.

The use of gold chloride is a step towards improving reaction results and increasing contrast. The process is supposed to be approximately as follows:

$$3\ Ag + AuCl_3 \rightarrow Au + 3\ AgCl$$

Through the use of gold chloride, silver is converted to silver chloride and metallic gold deposits in place of the silver. The gold deposit is more stable and more durable.

Treatment of the cells with gold chloride solution yields a clearer visualization, nonspecific reactions are reduced, and the brown coloration diminishes. The use of gold chloride solution increases the stability and durability of the stain; silver chloride can be removed by treating with sodium thiosulfate. The intensification of the reaction is probably due to a reduction of undesired, nonspecific argyrophilia of the background.

Ordering information

Name	Packaging	Ord. No.
Methenamine silver plating kit acc. to Gomori	7 x 100 ml, 10 tabs	1.00820.0001
Kit components		
Reagent 1: Periodic acid solution	100 ml	
Reagent 2: Silver nitrate solution	3 x 100 ml	
Reagent 3: Methenamine borate tablets	10 tablets	
Reagent 4: Gold chloride solution	100 ml	
Reagent 5: Sodium thiosulfate solution	100 ml	
Reagent 6: Light green SF solution	100 ml	

Warthin-Starry silver plating

Kit for detection of *Helicobacter pylori* in paraffin sections

In the Warthin-Starry silver staining method, silver nitrate is reduced with hydroquinone to metallic silver; the hydroquinone oxidizes to quinone. The reaction solution is selected such that the metallic silver is deposited in a specific reaction on the target structures, here *Helicobacter pylori.* The reaction is stopped by a rinsing step that terminates silver development. Silver is deposited on the surfaces of the bacteria, which appear dark brown to black under the microscope; cells turn yellow to golden yellow, while the nuclei are brown. The bacteria are found in the mucus of the surface epithelium, in the apical glands of the stomach and in the foveoles of the gastric mucosa. In a further step with sodium thiosulfate solution the deposited silver is fixed and stabilized. Use of sodium thiosulfate means that the specimen can subsequently be mounted with any xylene-containing mounting medium. If it is preferred not to use this additional staining bath, the slides must be mounted with DPX new to stop them fading.

Material

3-5 µm thick paraffin sections, formalin-fixed.

Preparation

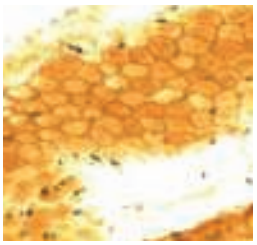
Application takes place in the jar. The kit is designed for a volume of 60 ml, which corresponds to a Hellendahl jar. Preheat the water bath up to 60 °C.

1. Production of diluted acetic acid solution

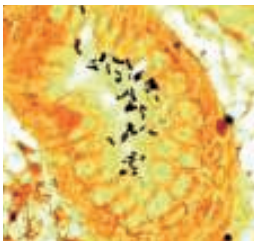
Acidify 1 l distilled water with 10 ml 1.2 % acetic acid (stock solution, can be stored for max. 3 weeks).

2. Impregnation solution

Add 50 ml diluted acetic acid solution + 10 ml 6 % silver nitrate solution, stir and place covered in the hot water bath and heat up to 60 °C. Check of temperature (ready-to-use solution).



Stomach biopsy, paraffin section, Warthin-Starry silver plating kit, modified



Stomach biopsy, paraffin section, Warthin-Starry silver plating kit, modified

Important
Do not use any metal things.

3. Developer solution

A) Preparation
Add 60 ml diluted acetic acid solution + 2 red dosage spoons of gelatine and place covered in the hot water bath at the same time as the impregnation solution and stirring occasionally. The gelatine must be completely dissolved. Further additions follow only just prior to placing the sections into this solution. (see Finishing)

B) Finishing
Add 2 orange-colored spoons (located in the cap; full to the brim) of hydroquinone mixture, stir well. Add 3 ml hot impregnation solution with the enclosed pipette, mix well.

Note
The sections must be placed immediately into the ready-to-use developer solution!

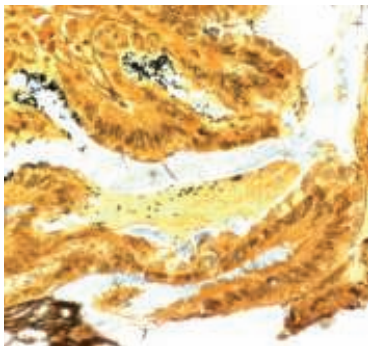
4. Distilled water
2 x rinse.

Coverslipping with DPX new
Coverslip the xylene-dampened preparations only with DPX new and the cover glass in order to keep the staining result stable.

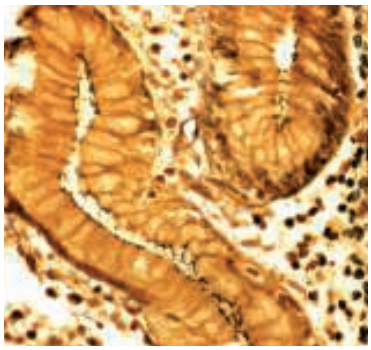
Protocol Warthin–Starry silver plating kit, modified

Step	Temperatur	Time
Deparaffinize the sections in the typical manner and rehydrate		
Distilled water		10 sec
Impregnation solution	60 °C	30 min
Rinse well in running tap water		3 min
Developer solution	60 °C	2-3 min
Distilled water	60 °C	10 sec
Distilled water	60 °C	2 min
Dehydrate and clarify sections in the typical manner		
Coverslipping with DPX new		

Result	
Helicobacter pylori	dark brown to black
Background	yellow to gold-yellow



Stomach biopsy, paraffin section, Warthin-Starry silver plating kit, modified



Stomach biopsy, paraffin section, Warthin-Starry silver plating kit, modified

Coverslipping with xylene-containing mounting agent

If another xylene-containing mounting agent is used, then an additional step with sodium thiosulfate solution must be taken to prevent non-specific silver deposits and keep the staining result stable.

Protocol Warthin-Starry silver plating kit, modified

Step	Temperatur	Time
Deparaffinize the sections in the typical manner and rehydrate		
Distilled water		10 sec
Impregnation solution	60 °C	30 min
Rinse well in running tap water		3 min
Developer solution	60 °C	2-3 min
Distilled water	60 °C	10 sec
Distilled water	60 °C	2 min
Sodium thiosulfate solution		3 min
Distilled water		10 sec
Dehydrate and clarify sections in the typical manner		
Coverslipping with xylene-containing mounting agent		

Result	
Helicobacter pylori	dark brown to black
Background	brownish

Ordering information

Name	Packaging	Ord. No.
Warthin-Starry silver plating kit, modified	for 500 tests	1.02414.0001
Kit components		
Reagent 1: Silver nitrate solution, 6 %	500 ml	
Reagent 2: Hydroquinone mixture	2 x 14 ml	
Reagent 3: Gelatine powder	130 g	
Reagent 4: Acetic acid solution, 1.2 %	60 ml	
Additional required		
DPX new	500 ml	100579
Sodium thiosulfate solution, 0.1 mol/l (0.1 N) Titrisol®	1 l	109950


25
20
15
10
5

Reticulin silver plating acc. to Gordon & Sweets

Reticular fibers that cannot be adequately visualized using an H&E stain may be rendered visible through a PAS reaction or, better, a silver plating stain. Reticulin visualization with a silver stain gives greater contrast and sharpness against the background than PAS. Reticular fibers consist, inter alia, of thin bundles of fine fibrils of type III collagen. These collagen fibrils react readily with silver salts and are therefore described as being argyrophilic and are stained black following silver plating. Reticular fibers can be visualized in basement membranes, supporting membranes around epithelial structures, capillaries and peripheral nerve fibers, as well as in organs such as the liver and kidneys and in particular in hematopoetic organs. There they form fiber meshworks and skeletons that provide flexible and elastic support for organs and tissues. Gordon & Sweets' silver stain for reticulin may be followed by nuclear staining with nuclear fast red-aluminum sulfate solution 0.1 % when nuclei, collagen and the background are to be stained in addition to the reticular fibers.

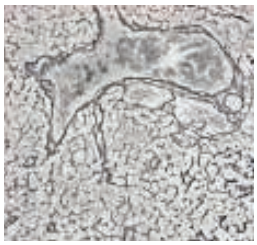
Procedure

Staining with the reagents 1-8 is performed on a staining rack made of plexiglass or coated with plastic. The reagents are dripped onto the section one after another so as to cover the section completely. Rinse with distilled water using a washing bottle. Do not use metal tweezers and do not allow any other metal objects to touch the section slides.

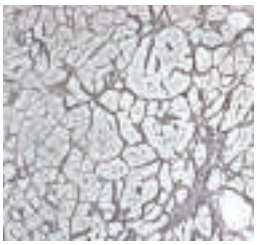
Protocol for Reticulin silver plating acc. to Gordon & Sweets

Step	Quantity	Time
Deparaffinize the sections in the typical manner and rehydrate		
Distilled water		10 sec
Drip reagent 1 and 2 one after the other	4 drops of each	5 min
Distilled water		10 sec
Reagent 3	8 drops	2 min
Distilled water		10 sec
Reagent 4	8 drops	2 min
Distilled water		10 sec
Reagent 5	8 drops	2 min
Distilled water		10 sec
Reagent 6	8 drops	2 min
Distilled water		10 sec
Reagent 7	8 drops	2 min
Distilled water		10 sec
Reagent 8	8 drops	2 min
Distilled water		10 sec
Dehydrate and clear the sections in the typical manner		
Mount the xylene-wet slides with Entellan® new or the Neo-Clear®-wet slides with Neo-Mount™		

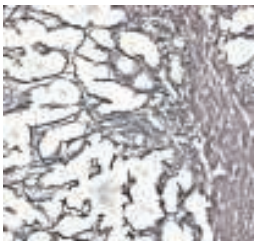
Result	
Reticular fibers	black



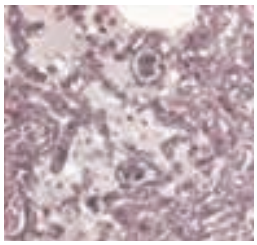
Lymph node, paraffin section, Reticulin silver plating kit acc. to Gordon & Sweets



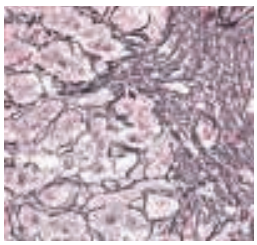
Pancreas, paraffin section, Reticulin silver plating kit acc. to Gordon & Sweets



Pancreas, paraffin section, Reticulin silver plating kit acc. to Gordon & Sweets



Lung, paraffin section, Reticulin silver plating kit acc. to Gordon & Sweets with nuclear fast red stain



Pancreas, paraffin section, Reticulin silver plating kit acc. to Gordon & Sweets with nuclear fast red stain

Protocol with Nuclear fast red-aluminum sulfate solution 0.1 %

Step	Time
Nuclear fast red-aluminum sulfate solution 0.1 %	3 min
Rinse with distilled water	10 sec
Dehydrate, clear and mount the sections in the typical manner	

Result	
Reticular fibers	black
Cell nuclei	red
Background	red
Collagen	red

Ordering information

Name	Packaging	Ord. No.
Reticulin silver staining kit acc. to Gordon & Sweets	2 x 16 ml, 6 x 30 ml	1.00251.0001
Kit components		
Reagent 1: Potassium permanganate solution	16 ml	
Reagent 2: Sulfuric acid	16 ml	
Reagent 3: Oxalic acid	30 ml	
Reagent 4: Ammonium iron(III) sulfate solution	30 ml	
Reagent 5: Ammoniacal silver nitrate solution	30 ml	
Reagent 6: Formaldehyde solution	30 ml	
Reagent 7: Gold chloride solution	30 ml	
Reagent 8: Sodium thiosulfate solution	30 ml	
Additional required		
Nuclear fast red-aluminum sulfate solution 0.1 %	500 ml	100121

Silver Plating Kit acc. to von Kossa

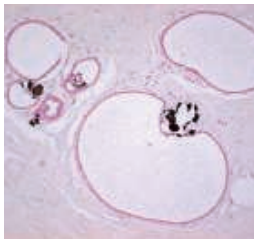
Kit for the detection of calcium deposits in paraffin sections

Silver plating methods are highly specific, They are used to visualize fine structures which have an affinity to silver ions. The so-called argentaffinen structures will be illustrated black by the reaction with silver. The Silver Plating Kit acc. to von Kossa is used to visualize calcium deposits in histological tissue specimens. Silver ions of the silver nitrate solution react with the carbonate and phosphate ions of the calcium and displace the calcium ions. These silver ions are reduced to metallic silver by exposure to strong light, and this silver is evaluated by microscopy. The silver staining methods could be difficult in application and require a particular accuracy.

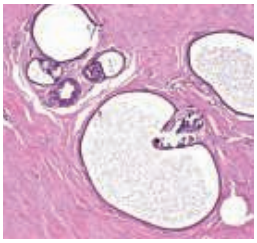
Procedure
The staining is carried out in Hellendahl cells.
The distance between the light source and the Hellendahl cell should be approx. 5 cm to ensure optimal exposure.

Additionally required auxiliaries for exposure
Light source (e.g. desk lamp) fitted with an energy-saving lamp of at least 20 watts.

Note:
Tissue specimens fixed with neutral buffered formalin.
Recommended section thickness 5-6 µm. Do not use metal tweezers and do not allow any other metal objects to come into contact with the slides.

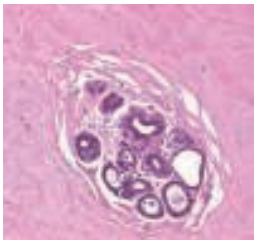


Paraffin section, mamma, Silver Plating acc. to von Kossa



Paraffin section, mamma, (identical sample) H&E

Paraffin section, mamma, Silver Plating acc. to von Kossa



Paraffin section, mamma, (identical sample) H&E

Protocol Silver Plating acc. to von Kossa

Step	Time
Deparaffinize the sections in the typical manner and rehydrate	
Rinse in distilled water	1 min
Silver nitrate solution under exposure to illuminant	20 min
Running tap water	3 min
Sodium thiosulfate solution	5 min
Running tap water	1 min
Nuclear fast red solution	3 min
Rinse in distilled water	1 min
Ethanol 70 %	1 min
Ethanol 96 %	1 min
Ethanol 100 %	1 min
Ethanol 100 %	1 min
Xylene or Neo-Clear®	5 min
Xylene or Neo-Clear®	5 min
Mount the xylene-wet slides with Entellan® new and the Neo-Clear®-wet slides with Neo-Mount™	

Result	
Calcium	brown to black
Cell nuclei	red
Background	red
Collagen	red

Ordering information

Name	Packaging	Ord. No.
Silver Plating Kit acc. to von Kossa	2 x 100 ml	1.00362.0001
Kit components		
Reagent 1: Silver nitrate solution	100 ml	
Reagent 2: Sodium thiosulfate solution	100 ml	
Additional required		
Nuclear fast red-aluminum sulfate solution 0.1 %	500 ml	100121

Congo red acc. to Highman

Kit for detection of amyloid

Besides the Highman method, another way of visualizing amyloid is with the methods according to Bennhold and Puchtler. The detection of amyloid is relevant for a whole group of diseases, some hereditary, such as Alzheimer's disease, Creutzfeld-Jakob's disease, bovine spongiform encephalopathy, myopathies and angiopathies, among others. Congo red staining is based on the formation of hydrogen bridge bonds with the carbohydrate component of the substrate. Amyloid that has been stained with congo red shows notable dichroism in polarized light, with deposits showing bright green against a dark background, while other materials, such as collagen, do not show this effect. The birefringence of congo red-stained amyloid, which appears green under polarized light, has greatly improved the sensitivity of the method. The affinity of the various amyloid proteins to congo red can be influenced, and thus diagnostics facilitated, through pre-treatment measures. Congo red staining remains one of the routine staining techniques in pathology. To make the Congo red staining procedure easier and the results reproducible, a reliable staining kit has been developed based on Highman's stain. The staining kit contains congo red solution and KOH differentiation solution, both ready-to-use.

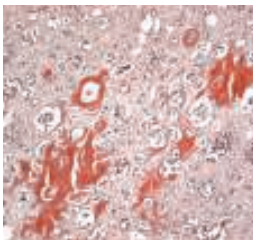
Material

In order best to demonstrate amyloid, it is important that the paraffin sections are not too thin; they should be 5-6 µm or preferably be 7 µm thick.

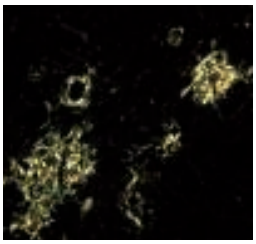
Protocol for Congo red staining kit acc. to Highman

Step	Concentration	Time
Deparaffinize the sections in the typical manner and rehydrate		
Rinse in distilled water		1 min
Stain in Hematoxylin solution acc. to Gill III		
Rinse under running tap water		5 min
Stain in Congo red solution		10 min
Rinse in running tap water		5 min
Differentiate in KOH solution		30-40 sec
Rinse in running tap water		5 min
Rinse in Ethanol	96 %	30 sec
Dehydrate in Ethanol	100 %	1 min
Dehydrate in Ethanol	100 %	1 min
Clear with xylene or Neo-Clear®		5 min
Clear with xylene or Neo-Clear®		5 min
Mount the xylene-wet slides with Entellan™ new or the Neo-Clear®-wet slides with Neo-Mount™		

Result	
Nuclei	dark blue
Amyloid in transmitted light	pink to red
Amyloid in polarized light	green metachromasia
Connective tissue	light red



Liver, paraffin section
Congo red staining kit
acc. to Highman, normal light



Liver, paraffin section
Congo red staining kit
acc. to Highman, polarized light

Positive control

In order to identify amyloid with certainty, the findings under transmitted light should always be followed by an inspection of the material under polarized light.

The microscope used should meet the requirements of a medical diagnostic laboratory and be equipped with a polarization facility.

Ordering information

Name	Packaging	Ord. No.
Congo red kit acc. to Highman	3 x 100 ml	1.01641.0001
Kit components		
Congo red solution	100 ml	
KOH solution	2 x 100 ml	
Additional required		
Hematoxylin solution modified acc. to Gill III	500 ml, 1 l, 2.5 l	105174

Tb-color modified

Kit for hot staining acid-fast bacteria

Due to the high proportion of wax and lipids in the cell wall, mycobacteria absorb dyes only very slowly. To accelerate the uptake of fuchsin dye and thus also the formation of the mycolate-fuchsin complex in the cell wall, the carbolfuchsin solution applied to the specimen is normally heated until vapors evolve. Once the mycobacteria have taken up the dye, they hardly release it again, even when they are intensively treated with a decolorizing solution such as hydrochloric acid in ethanol. The mycobacteria are thus referred to as being acid- and alcohol-fast in staining operations and show up red on the microscope slide, while all non-acid-fast microorganisms adopt the color of the corresponding counterstain.

Use

For the microscopic investigation of mycobacteria.
The Tb-color modified staining kit is for use with the classic Ziehl-Neelsen hot staining procedure.
The solutions are also available separately in various package sizes.

Material

Paraffin section – approx. 5 µm and air-dried, heat-fixed smears of bacteriological material such as sputum, FNAB, lavages, imprints, effusions, pus, exudates, as well as liquid and solid cultures.

Histological sections

Deparaffinize the sections in the typical manner and rehydrate in a descending alcohol series.

Protocol for Tb-color modified staining on the staining rack

Step	Time
Cover specimens completely with Tb-color modified carbol-fuchsin solution. Carefully heat 3 times from below with a Bunsen burner to steaming and keep hot	5 min
Do not allow the stain to boil.	
Wash with tap water until no further color is given off. Cover completely with Tb-color modified hydrochloric acid in ethanol and, depending on the thickness of the specimen	15-30 sec
Wash immediately with tap water.	
Counterstain in Tb-color modified methylene blue solution	30 sec
Wash well with tap water	
Dehydrate histological specimens (ascending alcohol series, clear in xylene or Neo-Clear®) and mount with Entellan™ new or Neo-Mount™	

Result	
Acid fast bacteria	red
Background	light blue

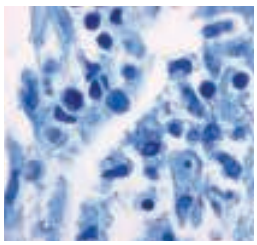
Assessment

A positive finding is reported as “acid fast bacteria detected” and a negative finding is reported as “acid fast bacteria not detected”.

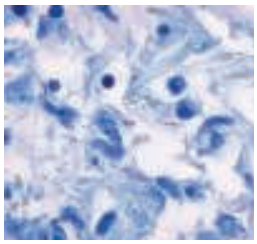
It is not possible to state whether there are tuberculosis bacteria or other “atypical” bacteria.

It is also impossible to state whether theses mycobacteria are still capable of reproduction or are already dead.

When acid-fast bacteria are found in the material examined, further investigations in a special laboratory are indicated.



Lung, paraffin section
Tb-color modified



Lung, paraffin section
Tb-color modified

Ordering information

Name	Packaging	Ord. No.
Tb-color modified staining kit	4 x 500 ml	1.00497.0001
Kit components		
Solution 1: Tb-color modified carbol-fuchsin solution	500 ml	
Solution 2: Tb-color modified hydrochloric acid in ethanol	2 x 500 ml	
Solution 3: Tb-color modified methylene blue solution	500 ml	
Single reagents		
Ziehl-Neelsen's carbol-fuchsin solution	100 ml, 500 ml, 2.5 l	109215
Löffler's methylene blue solution	500 ml, 2.5 l	101287
Hydrochloric acid in ethanol	1 l, 5 l	100327

TB-fluor

Kit for fluorescence staining acid-fast bacteria

Staining for fluorescence microscopy with Auramine O and Rhodamine B has become an established procedure alongside ZN staining, as fluorescence staining allows acid-fast rods to be easily identified even when their numbers are small. Depending on the filter combination in the microscope there is a choice between the two fluorescent dyes Rhodamine B – red and Auramine O – yellow-green. Potassium permanganate solution turns the background uniformly black in order to suppress troublesome intrinsic fluorescence of the specimen. If required, double staining with TB-color modified (ZN) is possible.

Sample material

Paraffin sections and heat-fixed smears of sputum, FNAB, lavages, imprints, body fluids, exudates, puss, liquid and solid cultures

Fixation

Fixation is carried out over the flame of a Bunsen burner (2-3 times, avoiding excessive heating). It is also possible to fix the smears in an oven at 100-110 °C for 20 min. Impairment of staining must be expected if a higher temperature or longer heating is employed.

Pretreatment

Sputum

Sputum should be pretreated with Sputofluol® in order to free the mycobacteria from surrounding mucus. The active ingredient in Sputofluol® is hypochlorite, which oxidatively dissolves the organic material while sparing the mycobacteria for the greater part.

In a centrifuge tube mix 1 part of the sample (at least 2 ml) with 3 parts of a 15 % Sputofluol® solution prepared with distilled water, and leave to react for 10 min shaking vigorously from time to time. Centrifuge at 3000-4800 U/min for 20 min, decant the supernatant, smear out the sediment allow to dry.

Punction and lavage material, sediments

After carrying out the appropriate enrichment measures smear out samples on the microscopic slides and allow to air dry.

Histological sections

Deparaffinize the sections in the typical manner and rehydrate in a descending alcohol series.

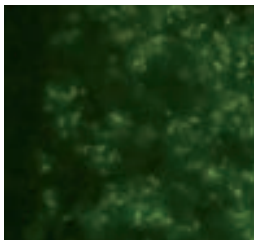
Protocol for TB-fluor in coplin jars

Step	Time
Stain with Auramine-Rhodamine solution	15 min
Rinse with tap water	10 min
Decolorisation solution	1 min
Rinse with tap water	5 min
Counterstaining with $KMnO_4$ solution	5 min
Rinse in distilled water	5 min
Dehydrate and clear in the typical manner. Mount with Neo-Mount™ or Entellan™ new	

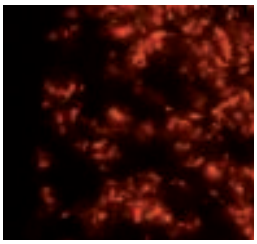
Protocol for TB-fluor in staining instruments

Step	Time
Stain with Auramine-Rhodamine solution	15 min
Rinse with tap water	10 min
Decolorisation solution	1 min
Rinse with tap water	5 min
Counterstaining with $KMnO_4$ solution	5 min
Rinse with tap water	5 min
Dehydrate and clear in the typical manner. Mount with Neo-Mount™ or Entellan™ new	

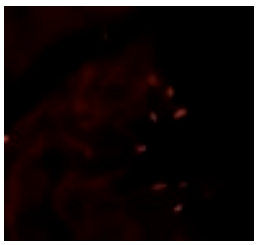
Result
Acid-fast bacteria appear red or yellow-green under the fluorescence microscope, depending on the filter combination used, against a dark background.



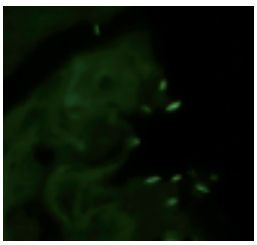
Lung, paraffin section
TB-fluor, Rhodamine staining



Lung, paraffin section
TB-fluor, Auramine staining



Lung, paraffin section
TB-fluor, Rhodamine staining



Lung, paraffin section
TB-fluor, Auramine staining

Interpretation

A positive reaction means “acid-fast-bacteria present”, while a negative reaction means “acid-fast-bacteria not present”. No distinction can be made regarding whether Mycobacterium tuberculosis or other mycobacteria have been detected, nor is it possible to ascertain whether the bacteria are alive or dead.

In the event of mycobacteria being found, further investigations should be conducted in special laboratories.

Technical requirements

Recommended filter combination for fluorescence microscopy:

Excitation filter	490-570 nm
Color separator	525 and 635 nm
Band elimination filter	505-600 nm

Ordering information

Name	Packaging	Ord. No.
TB-fluor staining kit	6 x 500 ml	1.09093.0001
Kit components		
Solution 1: Auramine-Rhodamine solution	500 ml	
Solution 2: Decolorisation solution	3 x 500 ml	
Solution 3: Counterstaining solution ($KMnO_4$)	2 x 500 ml	

TB-Fluor phenol-free

Kit for fluorescence staining acid-fast bacteria

Besides the TB-Fluor kit, the classic auramine-rhodamine stain, there is a kit with which the auramine-rhodamine staining solution is prepared without phenol. The necessary, efficient penetration of the dyes into the target structures is achieved through a phenol substitute that is more user- and eco- friendly than phenol. The TB-Fluor, phenol-free – is designed to be used for smears, though it can also be used for paraffin sections when steps are taken to ensure that the sections do not dry out on the staining bench.

Sample material

Heat-fixed smears of sputum, FNAB, lavages, imprints, body fluids, exudates, puss, liquid and solid cultures, histological sections.

Fixation

Fixation is carried out over the flame of a Bunsen burner (2-3 times, avoiding excessive heating). It is also possible to fix the smears in an oven at 100-110 °C for 20 min. Impairment of staining must be expected if a higher temperature or longer heating is employed.

Pretreatment

Sputum

Sputum should be pretreated with Sputofluol® in order to free the mycobacteria from surrounding mucus. The active ingredient in Sputofluol® is hypochlorite, which oxidatively dissolves the organic material while sparing the mycobacteria for the greater part.

In a centrifuge tube mix 1 part of the sample (at least 2 ml) with 3 parts of a 15 % Sputofluol® solution prepared with distilled water, and leave to react for 10 min shaking vigorously from time to time. Centrifuge at 3000-4800 U/min for 20 min, decant the supernatant, smear out the sediment allow to dry.

Punction and lavage material, sediments

After carrying out the appropriate enrichment measures smear out samples on the microscopic slides and allow to air dry.

Histological sections

Deparaffinize the sections in the typical manner and rehydrate in a descending alcohol series.

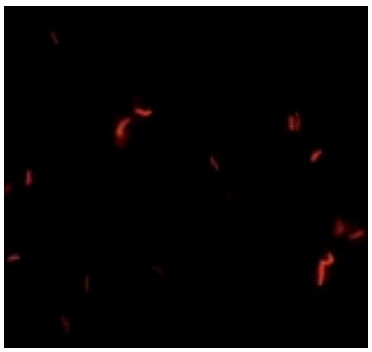
Protocol for TB-Fluor phenol-free on the staining rack

Step	Time
Cover the air-dried and heat fixed specimen completely with Auramine-Rhodamine staining solution and stain	15 min
Carefully rinse under running tap water	30 sec
Cover the specimens completely with decolorisation solution and leave to stand	1 min
Carefully rinse under running tap water	30 sec
Cover the specimens completely with $KMnO_4$ counterstaining solution and stain	5 min
Carefully rinse under running tap water	30 sec
Dehydrate and clear in the typical manner. Mount with Neo-Mount™ or Entellan™ new	

Result

Acid-fast bacteria appear red or yellow -green under the fluorescence microscope, depending on the filter combination used, against a dark background.

A positive result means "acid fast bacteria detected" and a negative result "acid fast bacteria not detected". It is impossible to say whether these rods are tubercle bacilli or other "atypical" mycobacteria or whether they are still capable of reproduction or already dead. In the event of mycobacteria being found, further investigations should be conducted in special laboratories.



Lung, paraffin section
TB-Fluor phenol-free, Rhodamine staining

Double staining

Any doubtful or suspicious result can be confirmed by conduction the double staining method "TB-Fluor-Tb-color or TB-Fluor-Tb-color modified". In the case of the un-mounted specimens stained with Tb-fluor, first only immersion oil for diagnostic purposes is used. Subsequently the immersion oil is carefully removed and the dried specimens are stained with Tb-color or Tb-color modified. The mycobacteria show up red against light green (Tb-color) or light blue (Tb-color modified) background. Histological sections can be treated in the same way with Tb-color modified, the coverglass must be removed before the second stain, stain acc. to the protocol.

Technical requirements

Recommended combination of filters for fluorescence microscopy:

Excitation filter	490-570 nm
Color split	525 and 635 nm
Suppression filter	505-600 nm

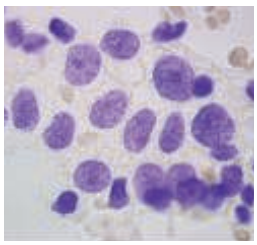
Ordering information

Name	Packaging	Ord. No.
TB-Fluor phenol-free	4 x 200 ml	1.01597.0001
Kit components		
Solution 1: Auramine-Rhodamine solution, phenol-free	200 ml	
Solution 2: Decolorisation solution	2 x 200 ml	
Solution 3: Counterstaining solution ($KMnO_4$)	200 ml	

DNA staining acc. to Feulgen

Kit for quantitative determination of DNA

The standardized Feulgen reaction is the method of choice for determining DNA in histological and cytological specimens. Ever since the significance of DNA content in diagnosis and prognosis, particularly with respect to undifferentiated tumors, was recognized, DNA image analysis has really come to the fore. A crucial factor in the reliability of DNA measurements is the reproducibility of the Feulgen reaction. Provided the staining directions are followed to the letter, the specially matched reagents contained in the Feulgen kit provide this necessary reproducibility.



Prostata, paraffin section
DNA staining kit acc. to Feulgen

Material

Specimens include monolayers of cell cultures, imprint smears, smears of fine needle aspiration biopsy (FNAB) material and exfoliative preparations, cytocentrifugates of ascites, pleural effusions, urine and CS fluid and, just in exceptional cases, sections of formalin-fixed, paraffin-embedded tissue.

Pretreatment

Sodium disulfite wash solution

To prepare approx. 100 ml of solution, mix together 95 ml of distilled water, 5 ml of sodium disulfite concentrate (Reagent 3) and 1 ml of HCl (Reagent 1).

Preparation of specimens

Various methods can be used to fix the specimen.

Gynecological smears

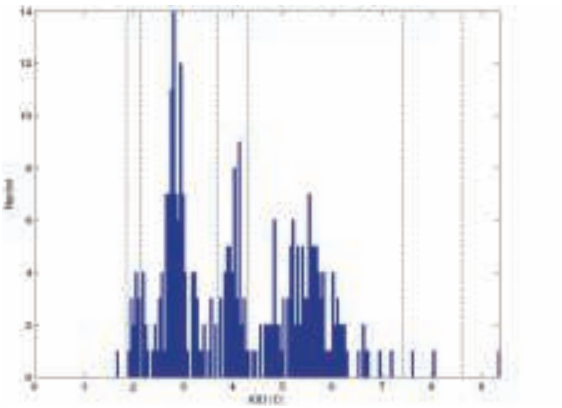
Step	Time
Spray fixing with M-FIX™	
Fix in phosphate-buffered (pH = 7.0) 4 % formaldehyde	1 hour
Rinse under running tap water	10 min

Other cytological specimens

Step	Time
Dry for one hour in air	
Fix in phosphate-buffered (pH = 7.0) 4 % formaldehyde	1 hour
Rinse under running tap water	10 min

Routine formalin-fixed, paraffin-embedded tissue sections

Step	Time
Deparaffinize the sections in the typical manner and rehydrate	
Rinse under running tap water	10 min



Histogram of the specimen, Prostata carcinoma G3

Procedure for measuring prepared specimens

Before being measured, the mounted specimens should be kept for 24 hours in the dark, as the refractive index of the mounting medium still changes for a time, which would compromise the reproducibility of the measurement.

Protocol for DNA staining acc. to Feulgen

Step	Concentration	Time
HCl at 22 °C (± 0.5 °C)		50 min
Distilled water		2 min
Distilled water		2 min
Schiff's reagent at room temperature		60 min
Sodium bisulfite washing solution		3 min
Sodium bisulfite washing solution		3 min
Distilled water		2 min
Distilled water		2 min
Ethanol	50 %	1 min
Ethanol	70 %	1 min
Ethanol	80 %	1 min
Ethanol	99 %	1 min
Xylene		1 min
Mounting with Entellan™ new and cover-glass		

Result	
Cell nuclei	red violet colored
Cytoplasm and background should be un-colored	

Ordering information

Name	Packaging	Ord. No.
DNA staining kit acc. to Feulgen	5 x 250 ml	1.07907.0001
Kit components		
Reagent 1: 5 M Hydrochloric acid	2 x 250 ml	
Reagent 2: Schiff's reagent	2 x 250 ml	
Reagent 3: Sodium metabisulfite concentrate	250 ml	

HEMATOGNOST Fe™

Kit for detection of free ionic iron by the Prussian blue reaction in histology and hematology

The Prussian blue reaction

In the Prussian blue reaction any free ferric ions (Fe^{3+}) in the tissue react with hexacyanoferrate (II) in aqueous hydrochloric acid solution. The reaction product is a typical bright blue precipitate that settles out in the target structure.

The reaction equation is as follows:

$$4\ Fe^{3+} + 3\ K_4Fe(CN)_6 \rightleftharpoons Fe_4(Fe(CN)_6)_3 + 12\ K^+$$

The Prussian blue reaction in histology

In histology, Prussian blue reactions are used, for instance, to diagnose hemochromatosis in the liver and to diagnose diseases of the lungs (asbestosis) and joints.

The Prussian blue reaction in hematology

In hematology, iron reactions are used in bone marrow diseases and especially in myelodysplastic syndrome (MDS).

Material

Paraffin sections 5-6 µm.

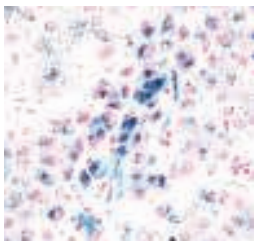
Preparation

Preparation of the staining solution for sections and smears

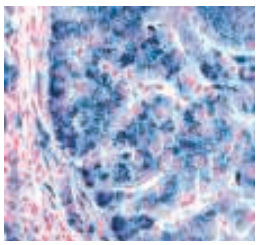
Reagent 1 and Reagent 2 are mixed in equal volumes. When 60 ml Hellendahl jar (with extensions) are used, a volume of 30-40 ml each of Reagent 1 and Reagent 2 is recommended. The staining solution should be discarded after each staining procedure.

Reagent 3: In order to achieve maximum optical differentiation of iron precipitates from cellular background, nuclear fast red solution is used giving a slightly pink counterstain. If more detailed information on cell morphology and structural elements is necessary or desired, counterstaining with the established “classical” procedures (Giemsa, May-Grünwald, hemalum, etc.) is also possible.

HEMATOGNOST Fe™ staining can also be carried out (without steps 4 and 5) on slides that have already been stained using one of the above mentioned methods.



Liver, paraffin section HEMATOGNOST Fe™, hemochromatosis – light level



Liver, paraffin section HEMATOGNOST Fe™, hemochromatosis – strong level

Information on pathological changes in cellular iron content

Increased levels of serum ferritin indicate hemochromatosis. Where this is suspected, confirmation must be provided through liver biopsy with determination of the liver iron content. Often a semiquantitative determination by the Prussian blue reaction is sufficient. Predominantly affected are the parenchyma and epithelial cells of the cystic duct. In tissue sections amounts of down to 0.002 µg ionic iron can be detected.

Protocol for HEMATOGNOST Fe™

Tissue sections*	Time
Deparaffinize the sections in the typical manner and rehydrate	
Place sections in freshly prepared staining solution and incubate	20 min
Rinse in distilled water	
Place sections in reagent solution 3 and incubate	5 min
Rinse briefly with distilled water	
Dehydrate sections with ascending ethanol series and clarify with xylene	
Cover with Entellan™ new	

Result	
Precipitates with Fe 3+ liberated from ferritin and haemosiderin	blue
Nuclei	pink or red

Ordering information

Name	Packaging	Ord. No.
HEMATOGNOST Fe™	4 x 250 ml	1.12084.0001
Kit components		
Reagent 1: Potassium hexacyanoferrate (II) solution	250 ml	
Reagent 2: HCl solution	250 ml	
Reagent 3: Nuclear fast red solution	2 x 250 ml	

LEUCOGNOST® NASDCL

Kit for the detection of specific esterases in immature and mature granulocytic cells

LEUCOGNOST® NASDCL allows specific esterases in immature cells of the granulocytic series in particular to be detected in paraffin sections and blood and bone marrow smears through an intense red coloration produced in an enzyme cytochemical reaction.

Material

Paraffin section 2-3 µm thick.

Preparation of the staining solution*

Solution A

Dilute 10 ml of Reagent 1 with 60 ml of distilled water, add 1 bottle of Reagent 2, and rewash the tube 2-3 times with a few ml of buffer.

Solution B

Add 15 drops of Reagent 3 to one bottle of Reagent 4, mix and allow to incubate for 2 minutes.

Solution C

Add Solution B to Solution A, and rewash the tube 2-3 times with a few ml of substrate buffer mixture.

* Prepare the solution immediately before using.

Preparation of the specimen

- Place 2-3 µm thick paraffin sections on coated microscope slides
- Dry the paraffin sections for 20 min at 60 °C and deparaffinize
- Allow the sections to dry for 30 min at room temperature
- Warm LEUCOGNOST® NASDCL to room temperature

Protocol for LEUCOGNOST® NASDCL

Tissue sections	Time
Deparaffinize the sections in the typical manner and rehydrate	
Incubate the sections in the freshly prepared staining solution at room temperature	30 min
Place in distilled water	5 min
Counter-stain in Mayer's hemalum	5 min
Rinse with tap water	5 min
Dehydrate and clear in the typical manner. Mount with Entellan® new	5 min

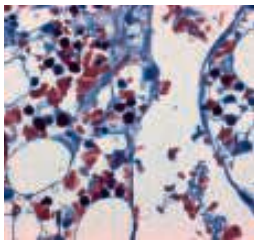
Result	
NASDCL positive cells	shining red
Background	blue
Tissue stem cells react positively	

Ordering information

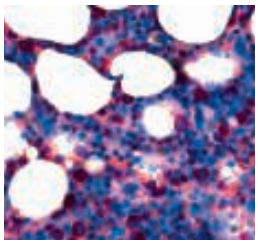
Name	Packaging	Ord. No.
LEUCOGNOST® NASDCL	for 12 staining batches (with up to 16 slides each)	1.16198.0001

Kit components

Reagent 1: Tris buffer concentrate
Reagent 2: Naphthol AS D chloroacetate
Reagent 3: Sodium nitrite solution
Reagent 4: Fast Red violet LB salt solution



Illiac crest puncture, LEUCOGNOST® NASDCL stain



Illiac crest puncture, LEUCOGNOST® NASDCL stain

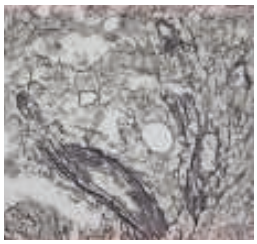
Users derive numerous benefits from employing these products for their daily routine and special applications.
A summary is presented here:

- Reproducible results
- Batch-to-batch consistency as a result of manufacturer's quality testing
- Simple, safe procedure
- Time saving during preparation
- Proven staining protocols based on international standards
- Certificates of analysis available for every batch
- Less direct contact with hazardous reagents/solid dyes
- The kit contains all of the reagents needed for the stain
- User-friendly and economic package sizes
- Developed, tested and produced in accordance with DIN ISO 9001
- Tested in accordance with DIN ISO 13485
- Products meet the IVD product requirements and are CE-certified

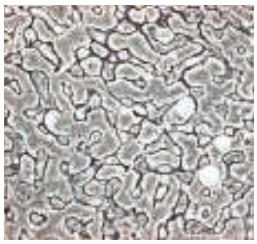
Benefits of ready-to-use solutions

ISOSLIDE®

Control slides for histological routine and special stains



ISOSLIDE® Reticulin control slide, vertebral body section, Reticulin silver plating



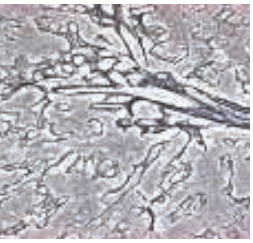
ISOSLIDE® Reticulin control slide, liver section, Reticulin silver plating

To ensure that the test preparations contain the material relevant for diagnostics and the target structures can be visualized by staining with good differentiation, it is recommended to use a control preparation. ISOSLIDE® control preparations are a group of products created to help optimize and standardize the staining result by enabling a direct comparison of typical control material with laboratory-internal specimens.

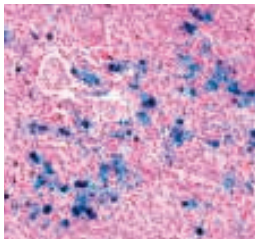
The ISOSLIDE® control slides consist of microscope slides with a paraffin section containing the typical structures of the test method to be used. The control slide is prepared using sections of suitable animal material. Each pack of ISOSLIDE® control slides contains 25 slides. One slide is stained with the reference method and is used for comparison. The 24 unstained slides are stained according to the corresponding Merck specification or else can be stained according to the laboratory-internal specification. The unstained control slides are stained and subsequently compared with the laboratory material and the stained control section regarding the staining result.

Material

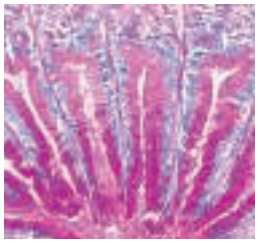
Paraffin sections approx. 3-4 µm from tissue samples of animal origin. The tissue is fixed with neutral buffered formalin.



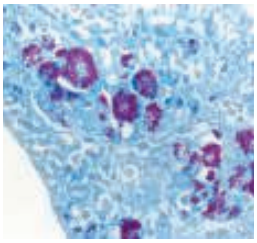
ISOSLIDE® Reticulin



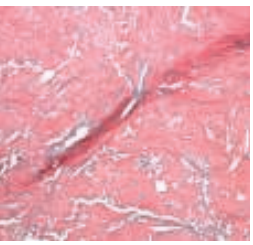
ISOSLIDE® Iron



ISOSLIDE® PAS

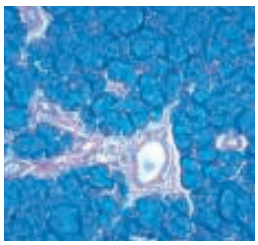


ISOSLIDE® AFB

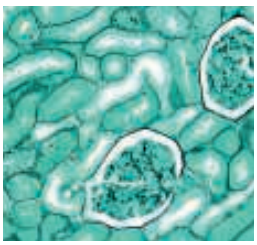


ISOSLIDE® Congo red (Light microscopy)

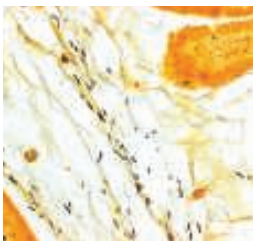
ISOSLIDE® Congo red (Polarised light)



ISOSLIDE® Alcian blue



ISOSLIDE® Methenamine



ISOSLIDE® Warthin-Starry

Name	Intented Use	Packaging	Ord. No.
ISOSLIDE® Reticulin	Control Slides with reference tissue for the detection of reticular fibers in histological tissue, 24 unstained paraffin sections, 1 stained control slide with Reticulin silver plating acc. to Gordon & Sweets, Cat. No. 100251	25 cotrol slides	1.00361.0001
ISOSLIDE® Iron	Control Slides with reference tissue for the detection of free ionic iron in histological tissue, 24 unstained paraffin sections, 1 stained control slide with HEMATOGNOST™ Fe® staining kit, Cat. No. 112084	25 cotrol slides	1.00380.0001
ISOSLIDE® PAS	Control Slides with reference tissue for the PAS staining in histological tissue, 24 unstained paraffin sections, 1 stained control slide with PAS staining kit, Cat. No. 101646	25 cotrol slides	1.00408.0001
ISOSLIDE® Alcian blue	Control Slides with reference tissue for the Alcian blue staining in histological tissue, 24 unstained paraffin sections, 1 stained control slide with Alcianblue-PAS, Cat. No. 101647 and 101646	25 cotrol slides	1.00425.0001
ISOSLIDE® Methenamine	Control Slides with reference tissue for the detection of argent-affin structures in histological tissue, 24 unstained paraffin sections, 1 stained control slide with Methenamine silver plating kit, Cat. No. 100820	25 cotrol slides	1.02473.0001
ISOSLIDE® Warthin-Starry	Control Slides with reference tissue for the detection of Helicobacter pylori in histological tissue, 24 unstained paraffin sections, 1 stained control slide with Warthin-Starry siver plating kit modified, Cat. No. 102414	25 cotrol slides	1.02472.0001
ISOSLIDE® AFB	Control Slides with reference tissue for the detection of Mycobacteria in histological tissue, 24 unstained paraffin sections, 1 stained control slide with TB-color modified Cat. No. 100497	25 cotrol slides	1.02560.0001
ISOSLIDE® Congo red	Control Slides with reference tissue for the detection of amyloid in histological tissue, 24 unstained paraffin sections, 1 stained control slide with Congo red staining acc. to Highman, Cat. No. 101641	25 cotrol slides	1.02561.0001

Mounting

Long-life slides for histology are mounted using mounting media that are initially applied to the slide in liquid form as viscous clear liquids and, as xylene, toluene or xylene substitute evaporate, remain as a hard, clear film between the slide with attached specimen and the coverslip.

Procedure

Histological and cytological specimens must be completely dehydrated before they are mounted. In the last step they are clarified with xylene or a xylene substitute to stop traces of water causing turbidity.
Approx. 0.5 ml of mounting medium is dropped onto a horizontally placed slide so as to fill the space between the slide and the coverslip. As soon as even distribution over the entire specimen is assured, a clean coverslip is carefully applied in such a way as to exclude air bubbles. Before it can be placed under the microscope, the slide is left in the horizontal position to dry for approx. 30 minutes. Complete drying, required before the slide can be archived, takes several hours. Slides prepared in this way remain color-fast for at least 5 years.

Anhydrous mounting media include classical products such as Canada balsam or modified products, generally acrylic resin mixtures, that are dissolved in aromatic solvents such as xylene or toluene.

Laboratories processing larger quantities of specimens are increasingly replacing manual mounting with automatic mounting using automatic coverslippers. This optimizes and standardizes the mounting process.

We have developed Entellan™ new for automatic coverslippers, a mounting medium with a narrow viscosity range specially designed for the purpose. Its viscosity ranges between 500 and 600 mPas. The narrow viscosity range improves and accelerates the application as neither the dropping rate nor the volume need any longer to be reset after the first run when a new bottle of Entellan™ new for automatic coverslippers is used.

Entellan™ new for automatic coverslippers is suitable for commercial coverslippers that work with glass coverslips. The mounting medium Entellan™ new for coverslippers is used as instructed in the coverslipper manual. The optimum quantity of mounting medium is calculated in an initial run with blank coverslips and slides based on the size of the coverslip and the size/thickness of the section, and checked when the bottle is changed.
DPX new is a water free mountimg medium for microscopy that substitute the previously used mounting medium DPX in all applications. Although DPX new still contains Xylene, the teratogenic ingredient Dibutyl phthalate (DBP) is missing, thus, its new composition is not only environmentally more beneficial, but also more user-friendly.
A new group of mounting media are those manufactured with solvents based on mixtures of aliphatic hydrocarbons.
Neo-Mount™ is a water-free mounting medium that has to be used following Neo-Clear® for mounting with coverslips in order to achieve the desired optical properties. Neo-Mount™ is extremely color-stable.
Neo-Mount™ contains Neo-Clear®, the aromatic-free substitute for xylene from Merck Millipore. It has a refractive index of approx. 1.46 and produces a clear, hard film under the coverslip. It takes approx. 30 minutes to dry and thus compares well with xylene-based mounting media.

All mounting media must meet certain chemical and physical criteria

1. refractive index of approx. 1.5, equal to that of glass, so that the mounting medium is practically invisible between the microscope slide with specimen and the coverslip,
2. viscosity high enough for the solution to become homogeneously distributed and not immediately run off the edges,
3. very low intrinsic fluorescence so that the slide can, if required, be examined under a fluorescence microscope.

Classical mounting medium

Canada balsam for microscopy	Ord. No. 101691
Refractive index (20 °C)	1.515-1.530
Density (20 °C/4 °C)	0.980 g/cm³

Modified mounting media

with toluene

Entellan™ rapid mounting medium for microscopy	Ord. No. 107960
Refractive index (20 °C)	1.492-1.500
Density (20 °C/4 °C)	0.925-0.935 g/cm³
Viscosity (20 °C)	60-100 mPas
Fluorescence	</= 100 ppb

with xylene

Entellan™ new rapid mounting medium for microscopy	Ord. No. 107961
Refractive index (20 °C)	1.490-1.500
Density (20 °C/4 °C)	0.94-0.96 g/cm³
Viscosity (20 °C)	250-600 mPas

Entellan™ new for cover slipper for microscopy	Ord. No. 100869
Refractive index (20 °C)	1.490-1.500
Viscosity (20 °C)	500-600 mPas
Fluorescence	</= 250 ppb

DPX new, water-free mounting medium for microscopy	Ord. No. 100579
Refractive index (20 °C)	1,518-1,521
Viscosity (20 °C)	600-700 mPas

with Neo-Clear®

Neo-Mount™ anhydrous mounting medium for microscopy	Ord. No. 109016
Refractive index (20 °C)	1.43-1.46
Viscosity (20 °C)	300-650 mPas
Fluorescence	</= 250 ppb

Technical note

Neo-Mount™ should not be used together with xylene in the same protocol, as this results in incompatibilities between the aromatic solvent and the aliphatic solvent in Neo-Mount™, causing the slides to become cloudy and streaked.
Any excess Neo-Clear® should be allowed to run off prior to coverslipping, as otherwise air bubbles might form under the coverslip. This is accomplished by placing the dehydrated slides on filter paper for approx. 1 minute and then mounting in the usual manner. With automatic coverslippers the slide reservoir must be used without Neo-Clear® solvent as a sure means of avoiding air bubbles.

Ordering information

Name	Packaging	Ord. No.
Canada balsam	25 ml, 100 ml	101691
Entellan™ rapid mounting medium for microscopy	500 ml	107960
Entellan™ new rapid mounting medium for microscopy	100 ml, 500 ml, 1 l	107961
Entellan™ new for cover slipper for microscopy	500 ml	100869
DPX new, water-free mounting medium for microscopy	500 ml	100579
Neo-Mount™ anhydrous mounting medium for microscopy	100 ml, 500 ml	109016

Dyes in solid form

Numerous solid dyes are available for routine and special uses in histology. For each application there are protocols that enable the users to prepare the required stains from solid dyes in their own laboratories. Certain dyes can be used in combination to achieve the proven result.

Ordering information

Name	C.I.	Application	Packaging	Ord. No.
Acid fuchsin	42685	Plasma staining	25 g	105231
Acridine orange $ZnCl_2$	46005	Vital and fluorescence staining	25 g	115931
Alcian blue 8GX	74247	Visualisation of acid mucosubstances	10 g	105234
Brilliant green (hydrogene sulfate)	42040	Spore staining	25 g	101374
Brilliant cresyl blue $ZnCl_2$	-	Reticulocytes staining	25 g	101368
Carmine	75470	Histological sections	5 g, 25 g	115933
Cresyl violet acetate	-	Nuclear staining	25 g	105235
Crystal violet	42555	Gram staining	25 g, 100 g	115940
Eosin B	45400	Plasma staining	25 g, 100 g	115934
Eosin Y	45380	Plasma staining	25 g, 100 g	115935
Eythrosin B	45430	Plasma staining	10 g, 25 g	115936
Fast green FCF	42053	Staining testicle specimen	25 g	104022
Fuchsin (basic)	42510	Nuclear and bacteria staining	100 g	115937
Hematoxylin monohydrate	75290	Nuclear staining	25 g, 100 g	115938
Indigo carmine	73015	Connective tissue staining	25 g	104741
Light green SF	42095	Chromosomes	25 g, 100 g	115941
Malachite green (oxalate)	42000	Counterstaining	25 g, 100 g	115942
Methylene blue	52015	Bacteria and Counterstaining	25 g, 100 g	115943
Methyl green $ZnCl_2$	42590	Chromatin	25 g	115944
Methyl violet	42535	Amyloid	25 g	115945
Neutral red	50040	Brain sections	25 g	101376
New fuchsin	42520	Mycobacteria staining	100 g	105226
Nigrosin (water-soluble)	50420	Bacteria staining	25 g	115924
Nil blue (hydrogen sulfate)	51180	Liver sections	25 g	115946
Nuclear fast red	60760	Counterstaining	25 g	115939
Oil red O	26125	Fat staining	25 g	105230
Orange G	16230	Plasma staining	25 g	115925
Orceine (synthetic)	-	Elastic fibers	5 g, 25 g	107100
Pararosaniline (chloride)	42500	Aldehyde visualisation	25 g, 100 g	107509
Phloxin B	45410	Cytoplasm staining	25 g	115926
Ponceau S	27195	Connnective staining	25 g	115927
Pyronine G	45005	Methyl green-pyronine staining	5 g	107518
Safranin O	50240	Chromosom and Gram staining	25 g	115948
Thionine (acetate)	52000	Brain sections	25 g	115929
Toluidine blue O $ZnCl_2$	52040	Nuclear staining; Metachromasia	25 g	115930

Certistain®

The Certistain® group of dyes comprises more than 30 dyes for microscopy. The dyes are tested to ensure they meet the specified chemical and physical criteria, and a typical application test is performed for each dye. Certistain® stands for excellent and reproducible staining results. For every Certistain® dye there are directions for a typical application. The directions can be downloaded from www.merckmillipore.com and are also available on request.

Other dyes in solid form

Besides Certistain® there are numerous other microscopy dyes that are used for classical staining with user-prepared solutions. For special stains that are not often performed and for those where no commercially prepared solutions are available these dyes offer a viable alternative along with Certistain® dyes.

Ordering information

Name	C.I.	Application	Packaging	Ord. No.
Amido black 10 B	20470	Collagenic connective tissue	25 g, 100 g	101167
Auramine O	41000	Mycobacteria fluorescence staining	50 g	101301
Carminic acid	75470	Carmalum, paracarmine	1 g, 5 g	100211
4',6'-Diamidino-2-phenylindole-dihydrochloride (DAPI)	-	DNA Fluorescence staining (cryo sections)	100 g	124653
Fluorescein isothiocyanate FITC	-	Marking of proteines	250 g	124546
Giemsa's azure eosin methylene blue	-	Lymphatic specimen, detection of *Helicobacter pylori* in stomach biopsies	25 g, 100 g	109203
Hematein	75290	Nuclear staining	25 g	111487
Hematoxylin crystals	75290	Nuclear staining	25 g, 100 g	104302
Malachite green (oxalate)	42000	Counterstaining	25 g, 100 g	101398
Methyl blue (> 60 %)	42780	Connective tissue staining	50 g	116316
Rhodamine B	45170	Mycobacteria fluorescence staining	25 g, 100 g	107599

Quality management

Quality management requirements in the histology laboratory are met through the use of certified products and, in particular, through the use of ready-to-use staining solutions and staining kits.

Products that are designated as IVDs and thus undergo suitability testing for dedicated use in diagnostic applications and bear the CE mark which has been mandatory in Europe since the end of 2003 are appropriately documented. The documents provide users with important information for their everyday work and assure safety. CE marking is a selfcertification process documenting every step in the product cycle along with quality management details.

Documents that relate to the product, such as the batch-specific certificate of analysis, the safety data sheet, instructions for use and technical information sheets are available from our website, www.merckmillipore.com, or on request.

Every product will have been the subject of an ISO 13485 risk assessment to see where risks to users and other parties might exist during use of the products and what can be done to stop such situations occurring. The product instructions, which are an important element of the documentation, contain general information on the use of IVDs as well as specific product- and application-related information. IVDs undergo regular internal and external audits to check the accuracy and completeness of the documentation in all areas.

Any technical enquiries and complaints that are received concerning an IVD are processed using dedicated software and managed and saved in a database. Whenever a complaint is received, a check is carried out to see whether an RCA (Root Cause Analysis) with CAPA (Corrective Action Preventive Action) is required. The customer care system helps in the identification of any problems with the product or process and the seeking of an efficient solution.

The successful passage of products through the process vouches for reproducible results and dedicated use, providing users with product information as well a safety assurance for their daily work and assistance in the event of a problem occurring.

During accreditation of a laboratory, the documents relating to the products can help simplify the documentation of laboratory procedures. Everything is simpler when commercially manufactured products are used in the process so that as may steps in the laboratory as possible are covered by detailed documentation.

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References

- J. Bancroft, A. Stevens
  Theory and Practice of Histological Techniques,
  Churchill Livingstone, Edinburgh (1990)

- A. Böcking, F. Giroud, A. Reith
  Consensus report of the ESACP task force on
  standardization of diagnostic DNA image cytometry,
  Analytical Cellular Pathology 8, 65-74 (1995)

- G. Clark
  Staining Procedures,
  Williams & Wilkins, Baltimore (1981)

- H. J. Conn's
  Biological Stain's,
  Williams & Wilkins, Baltimore (1977)

- European Committee for Clinical Laboratory Standards
  (ECCLS) S on RM for TS [SRMTS] (1992)
  Dye Standards, Part I, Terminology and general principles,
  Histochemical Journal 24, 217-219

- R. W. Horobin, J. A. Kiernan
  Conn's biological Stains, A Handbook of Dyes,
  Stains and Fluorochromes for Use in Biology and Medicine,
  10th Edition, BIOS Scientific Publisher, Oxford UK (2002)

- J. A. Kiernan
  Histological & Histochemical Methods:
  Theory & Practice, 2nd Edition,
  Pergamon Press, Oxford (1990)

- D. P. Penney, J. M. Powers, M. Frank, C. Willis, C. Churukian
  Analysis and testing of biological stains –
  The Biological Stain Commission Procedures,
  Biotechnic & Histochemistry, 77 (586),
  237-275 (2002)

- M. Mulisch; U. Welsch (Hrsg.)
  Romeis Mikroskopische Technik,
  18. Auflage, Spektrum Akademischer Verlag (2010)

- L. Vacca
  Laboratory Manual of Histochemistry,
  Raven Press, New York (1985)

- D. Wittekind
  Standardization of Dyes and Stains for Automated Cell Pattern Recognition, Analytical and Quantitative Cytology and Histology, Vol. 7, No. 1, March 1985

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References

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