Stains
for Microorganisms

Reliable results with high quality products

Merck Millipore is a division of MERCK

Introduction

In bacteriology the use of stains is standard. The staining procedure is the first step in the diagnostic – the result is immediately available, the result gives important information, more staining procedures can be carried out on demand, further required tests can be added immediately. The diagnostic accuracy and sensitivity, easy application, the speed and suitability for a huge range of specimen, the cost effectiveness, the repeatability are the reason that the staining procedures have such an importance in bacteriology. The classic methods as Gram or Ziehl-Neelsen staining are available since more then 130 years and used nowadays with the same importance.

Modification of the basic methods are available now, which have to be sensitive and specific in the same way and offer besides more convenience for the user – Gram staining and Tb fluorescence staining without phenol or cold staining for Tb detection are examples and available at Merck Millipore. All the bacteriological products are regulated products and fulfil the status of IVD products. They can be used for routine applications in each lab. That means that the Sputofluol used for preatreatment of sputum, all the ready-to-use staining solutions, the dry dyes for self-preparation of staining solutions and the immersion media are IVD products according to the directive 98/79/EC from October 20th, 1998 for In-vitro-diagnostica (IVD) and bear the CE mark. They can be used for routine applications in each lab. Insert sheets for all the bacteriological IVD products are available in the internet or on demand.

Content

Fixing

Fixation of sample material means interruption of complex intra- and supravital metabolic processes, structure preservation and prevention of postmortal signs of disintegration.

Fixing is an important step to preserve the sample material and enable visualisation through staining.

Bacteria, fungi, spores, viruses, protozoa and any other specimen contents are still alive at the time of sample withdrawal. Consequently, the specimen may be highly infective. Effective and extensive fixation is, therefore, extremely important to protect the operators handling the sample material and to stop any vital processes in the specimen. Thus, the material is no longer infective and the results are not distorted by continued growth.

After adequate air-drying for 5 to 15 minutes depending on the material, heat or chemical fixing is carefully performed.

01

Heat fixing

Solution

Physiological sodium chloride solution: Dissolve 9 g sodium chloride in 1 litre of demineralized water.

Experimental procedure and application

Using a loop, place the specimen on a fat free slide and streak either directly (e.g. viscous exsudate, puss or liquid cultures) or after dilution with physiological sodium chloride solution (e.g. centrifuge sediment, pure cultures from solid culture media).
After complete air drying, or for the sake of speed after careful heat drying, fix by slowly moving the slide in a circle of about 2.5 cm three times through the dark flame of a Bunsen burner (the specime side of the slide should be at the top).

Ordering information

Product	Package size	Cat. No.
Sodium chloride for analysis EMSURE® ACS, ISO, Reag. Ph Eur	500 g, 1 kg	106404

Chemical fixing

In contrast to heat fixing, chemical fixing permits a better contrasted visualisation of bacteria details e.g. the cilia or the relationship between bacteria and body cells.

Solutions

1. Ethanol ether: Ethanol abs. 50 ml; diethyl ether 50 ml.
2. Sublimate alcohol: Mercury(II) chloride 3 g; demin. water to 60 ml; ethanol abs. 30 ml.
3. Osmic acid solution: Osmic acid solution 2% 5 ml; conc. acetic acid 5 drops. Store in a bottle with a wide neck.

Experimental procedure and evaluation

Cover specimens with the following fluids or lay specimens in the baths:

a) methanol 2–3 min
b) or ethanol ether (1) 10–15 min
c) or sublimate alcohol (2) 3–5 min
d) It is also possible to hold the slide above hot steaming water and then place the wet slide on the opening of the wide neck bottle (3) to impregnate it with osmic acid fumes.

Ordering information

Product	Package size	Cat. No.
Methanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	106009
Ethanol absolute for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100983
Diethyl ether for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100921
Mercury(II) chloride for analysis Reag. Ph Eur, ACS	50 g	104419
Osmic acid solution 2% for electron microscopy	5 ml	109266
Acetic acid 96% for analysis EMSURE®	1 L, 2.5 L	100062

Stains

02

In bacteriology, staining solutions often contained phenol to be able to visualise all bacteria with one and the same stain. Due to the diverse structures of their cell walls, bacteria present different staining behaviour. In Gram staining, which was developed in 1884, phenol is added to Gram's crystal violet solution. Gram staining is the standard staining procedure used in bacteriology as it rapidly classifies bacteria as either gram-positive or gram-negative. Crystal violet and phenol form a complex with iodine from Lugol's solution. Due to their special structure characterised by a thick bacterial cell wall containing many mesh-like layers of murein (polypeptidoglycan), gram-positive bacteria retain the dye-iodine complex throughout the destaining step. Gram-negative bacteria, which only possess one thin layer of murein (polypeptidoglycan), are decolorised and, then, counterstained with safranine or carbol-fuchsin solution (ZN).

The Ziehl-Neelsen stain (ZN) for the detection of mycobacteria is as old as the Gram stain. Reliable staining of mycobacteria is also performed with a phenolic staining solution made from basic fuchsin. The dye can, thus, permeate the mycolate layer of mycobacteria which retain the dye upon destaining. Mycobacteria are thus referred to as being alcohol- and acid-fast (acid-fast bacteria, AFB). Counterstaining is performed with methylene blue, which is also employed for overview staining.

Other dyes used for staining of bacteria, fungi and spores are malachite green, methyl green, pyronine, lactophenol blue, Giemsa stain, safranine, eosin Y, pararosaniline and fluorescent dyes such as Rhodamine and Auramine.

Methylene blue staining

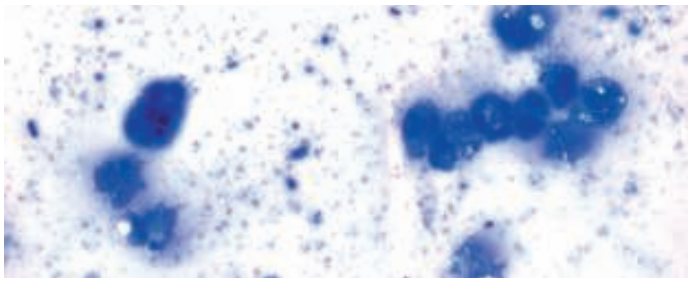
The methylene blue stain is an appropriate staining method to obtain a general picture e.g. of Gonococci, lactobacilli and to visualize polar bodies of Pasteurella.

Experimental Procedure and Application

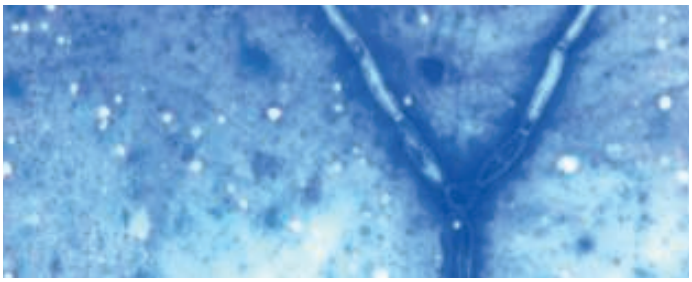
Stain fixed, air dried specimes for about 15 sec (thin smears) to 45 sec (thick specimens) with Löffler's methylene blue solution. Only stain Gonococci very briefly. If necessary differentiate with 0.5 to 1% acetic acid solution. Rinse with water and dry.

Ordering information

Product	Package size	Cat. No.
Löffler's methylene blue solution for microscopy	100 ml, 500 ml, 2.5 L	101287
Acetic acid 96% for analysis EMSURE®	1 L, 2.5 L	100062

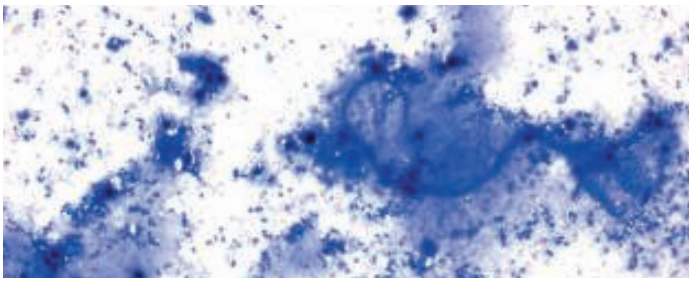


Mixed culture, methylene blue stain



Yeast, methylene blue stain

Culture, methylene blue stain



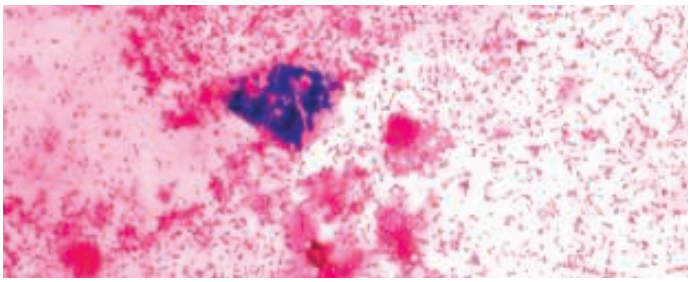
Mixed culture, methylene blue stain

Gram-staining

In the staining procedure aniline dyes are coupled with iodine to the bacterial cell wall to form a dye-iodine complex. All bacteria can be classified as Gram-positive or Gram-negative on the basis of this staining technique. In the case of Gram-positive organisms the dye-iodine complex cannot subsequently be dissolved from the cells with decolorizing agents such as alcohol or acetone; the cell remains blue-violet. In Gram-negative bacteria the dye-iodine complex is dissolved by these agents. The decolorated cells are then stained pink to red as a result of counterstaining with carbol-fuchsin, or orange with safranine.

Gram-staining (original method)

1) Cover the slide completely with crystal violet solution, stain for 3 min, pour off, do not rinse.
2) Cover the slide completely with Lugol's solution, leave for 2 min, pour off, do not rinse.
3) Immerse the slide completely in decolorizing solution (acetone, ethanol or methanol) and move for about 20–60 sec until no more clouds of stain are released and the smear is blue-grey.
4) Rinse carefully with distilled water for about 5 sec.
5) Cover the slide completely with Ziehl-Neelsen's carbol-fuchsin solution diluted 1:10, stain for 1 min, pour off.
6) Rinse carefully with distilled water for about 5 sec.
7) Allow to dry.



Culture, mostly negative rod, Gram-color

Results

Gram-positive bacteria: dark violet
Gram-negative bacteria: red

Gram-staining acc. to Hucker with Gram-color staining set (Staining rack)

1) Cover the fixed smear with crystal violet and stain for 1 min.
2) Pour off the crystal violet solution and rinse with Lugol's solution.
3) Cover with Lugol's solution and leave for 1 min.
4) Rinse with water.
5) Swirl in the decolorizing solution for 1 min.
6) Rinse with water.
7) Stain for 1 min in safranine solution.
8) Rinse with water and dry.

Results

Gram-positive bacteria: dark violet
Gram-negative bacteria: orange

Gram-staining acc. to Hucker with Gram-color staining set (automated jar staining)

1) Immerse the fixed smear for 3 min in crystal violet solution (dilute 1:5 with water).
2) 1 min in Lugol's solution.
3) 3 min in a second cuvette containing Lugol's solution.
4) 1 min in water.
5) 1 min in decolorizing solution.
6) 1 min in water.
7) 1 min in safranine solution.
8) 1 min in water (not necessary if the smear is immediately rinsed under running water).

Results

Gram-positive bacteria: dark violet
Gram-negative bacteria: orange

Ordering information

Product	Package size	Ord. No.
Gram-color		1.11885.0001
Kit components		
Solution 1: Gram's crystal violet solution	500 ml	
Solution 2: Lugol's solution stabilized	500 ml	
Solution 3: Gram's decolorizing solution	2 x 500 ml	
Solution 4: Gram's safranine solution	500 ml	
Single reagents		
Gram's crystal violet solution	500 ml, 2.5 L	109218
Gram's safranine solution	500 ml, 2.5 L	109217
Gram's decolorizing solution	500 ml, 2.5 L	110218
Lugol's solution stabilized with PVP	1 L, 2.5 L	100567
Lugol's solution (diluted iodine-potassium iodide solution) for the Gram staining method	250 ml, 1 L	109261
Ziehl-Neelsen's carbol-fuchsin solution	100 ml, 500 ml, 2.5 L	109215
Methanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	106009
Ethanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100983
Acetone for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100014

Gram-color modified, phenol-free

Principle

Aniline dyes in the cell tissue of microorganisms form a red dye-iodine complex when exposed to iodine. Sodium hydrogen carbonate enhances the formation of this complex further still. In Gram-positive microorganisms the dye-iodine complex cannot be subsequently dissolved from the cells by decolorizing agents such as alcohol or acetone. The cell remains dark blue in color. In Gram-negative microorganisms the dye-iodine complex is dissolved and the cell turns red as a result of counterstaining.

Preparing the smears

Apply the specimen material – for example body liquids, exsudates, puss, or a liquid or solid culture – to a degreased microscopic slide using an annealed loop. Subsequently distribute the specimen, either directly or after adding 1 to 2 drops of physiological saline solution, and smear out. Allow to air-dry and heat-fix the smear by slowly drawing the slide (with the smear side facing upwards) through the upper part of a Bunsen flame three times; subsequently allow to cool and then perform the staining procedure.

Preparing the staining solution

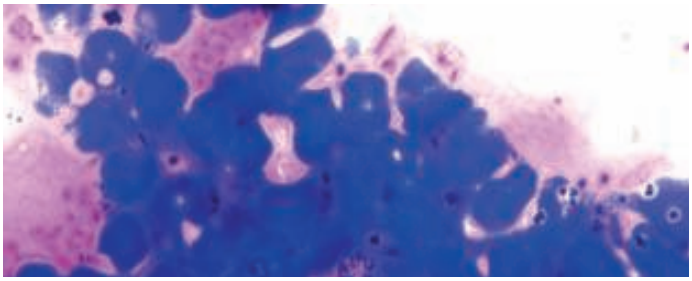
Mix the reagents crystal violet solution (solution 1a) and sodium hydrogen carbonate solution (solution 1b) 1:1 in the bottle provided (1c). This mixture is sufficient for approximately 65–70 specimens and can be stored at room temperature for 10 days and refrigerated 14 days, respectively. If this amount appears to be too large for this period of time, it is advisable to prepare a smaller quantity (approx. 3 ml per microscopic slide).

Procedure on the staining bench

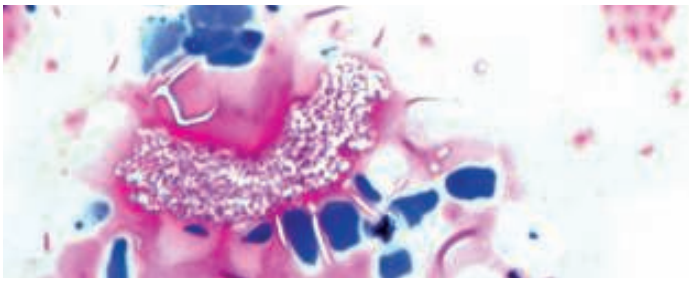
1)	Cover the microscopic slide completely with working solution 1c (mixture of crystal violet solution (1a) and sodium hydrogen carbonate solution (1b) 1:1) and stain.	1 min
2)	Carefully rinse with distilled water.	5 sec
3)	Cover the microscopic slide completely with solution 2, stabilized iodine solution.	1 min
4)	Carefully rinse with distilled water.	5 sec
5)	Decolor by covering completely with solution 3, decolorizing solution.	5–10 sec
6)	Carefully rinse with distilled water.	5 sec
7)	Counterstain by covering completely with solution 4, fuchsin solution.	15–30 sec
8)	Carefully rinse with distilled water.	5 sec
9)	Dry.	

Results

Gram-positive microorganisms: dark blue
Gram-negative microorganisms: red



Blood cultur, Gram-color modified



Mixed culture, Gram-color modified

For more working flexibility: Prepare the working solution with crystal violet and sodium hydrogene carbonate in a sufficient volume concerning to the slide number.

Storage

The staining kit must be stored at room temperature. Storage at temperatures below 15°C may result in a colored precipitate settling out of the staining solutions; in such a case the bottles should be conditioned by placing them in a water bath set at 60°C for 2–3 hours. This will redissolve the greater part of the dye precipitates. Subsequently filter the staining solutions through a paper filter.

Checking the staining kit

The staining kit can be checked using Gram-positive bacteria (staphylococci) and Gram-negative bacteria (Escherichia coli). Cultures taken from a culture medium incubated for 18–24 hours must be used for this purpose.

Ordering information

Product	Package size	Ord. No.
Gram-color modified, phenol-free		1.16301.0001
Kit components		
Number 1a: Crystal violet solution, phenol-free	100 ml	
Number 1b: Sodium hydrogen carbonate solution	100 ml	
Number 1c: Bottle for working solution of 1a und 1b		
Number 2: Iodine solution, PVP-stabilized	190 ml	
Number 3: Decolorizing solution	190 ml	
Number 4: Fuchsin solution, phenol-free	190 ml	
Auxiliary reagents		
Immersion oil	100 ml, 500 ml	104699
Immersion oil acc. to ISO 8036 modified	100 ml	115577
Oil of cedar wood	100 ml, 500 ml	106965
Entellan® new	100 ml, 500 ml, 1 L	107961
Ringer tablets	1 pack (100 tabl)	115525

Diphtheria bacteria staining acc. to Albert and Laybourn

Solutions

1. Toluidine blue malachite green solution: Toluidine blue 1.5 g; malachite green 2.0 g; dissolve in ethanol 20 ml; demineralised water 1 L; glacial acetic acid 10 ml; leave to stand for 1 to 2 days; filter.
2. Lugol's solution.

Experimental procedure and application

After heat fixing, stain the smear for 4–6 min with toluidine blue malachite green solution (1), rinse with water, cover with Lugol's solution for 2 min, rinse with water and dry between blotting paper.

Results

Polar bodies: blue-black
Bacteria body: green

Ordering information

Product	Package size	Cat. No.
Toluidine blue O (C.I. 52040) for microscopy Certistain®	25 g	115930
Malachite green oxalate (C.I. 42000) for microscopy Certistain®	25 g, 100 g	115942
Ethanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100983
Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100063
Lugol's solution stabilized with PVP	1 L, 2.5 L	100567
Lugol's solution (diluted iodine-potassium iodide solution) for the Gram staining method	250 ml, 1 L	109261

Gonococci staining acc. to Pappenheim-Unna

Solution

Carbol methyl green pyronine solution: Methyl green 0.1 g; pyronine 0.9 g; ethanol abs. 9 ml; glycerol 10 ml; phenol water 0.5% 100 ml; dissolve at about 50°C while shaking and leave to stand for 14 days.

Experimental procedure and application

Fix the air dried smear with methanol or ethanol ether and then stain for 2–5 min with the staining solution, rinse with demineralized water and dry.

Results

Nuclei: blue-green
Bacteria (Gonococci), fungi hyphae: ruby red
Plasma: light red

The color contrast makes it easier to find the Gonococci.
The coloring, however, is not specific for Gonococci!

Ordering information

Product	Package size	Cat. No.
Methyl green zinc chloride double salt (C.I. 42590) for microscopy Certistain®	25 g	115944
Pyronine G (C.I. 45005) for microscopy Certistain®	25 g	107518
Glycerol 85% for analysis EMSURE® Reag. Ph Eur	500 ml, 1 L	104094
Ethanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100983
Phenol GR for analysis ACS, Reag. Ph Eur	250 g, 1 kg	100206

Gonococci staining acc. to Schlirf

Solutions

1. Crystal violet solution: Crystal violet 4 g; ethanol abs. 100 ml; dissolve at 40 to 50°C, filter after cooling.
2. Methylene blue solution: Methylene blue 2 g; ethanol abs. 100 ml; dissolve at 40 to 50°C, filter after cooling.
3. Carbol crystal violet methylene blue solution: solution (1) 15 ml; solution (2) 10 ml, phenol water 2% 100 ml; demineralized water 50 ml. Filter before use.
4. Carbol methyl green pyronine solution: (Manufacture: see Gonococci staining acc. to Pappenheim-Unna). Before use, dilute 1+5.

Experimental procedure and application

Stain fixed smears for 1 min in staining solution (3), rinse with water, dry between blotting papier and rinse gently for 1 min with Lugol's solution. Differentiate with ethanol abs. for about 30 sec, restain with solution (4) for 2 min, rinse and dry.

Results

Gram-positive bacteria: black
Gonococci (Gram-negative): red

Ordering information

Product	Package size	Cat. No.
Crystal violet (C.I. 42555) for microscopy Certistain®	25 g, 100 g	115940
Methylene blue (C.I. 52015) for microscopy Certistain®	25 g, 100 g	115943
Lugol's solution stabilized with PVP	1 L, 2.5 L	100567
Lugol's solution (diluted iodine-potassium iodide solution) for the Gram staining method	250 ml, 1 L	109261
Ethanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100983
Phenol GR for analysis ACS, Reag. Ph Eur	250 g, 1 kg	100206

Enrichment of the Tb examination material

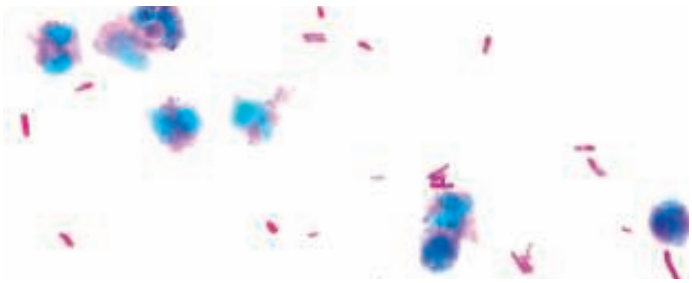
For the oxidative dissolution of organic material (cell material, mucus, etc.) in order to liberate tubercle bacilli from sputum and other material.

Mode of action

If tubercle bacilli are to be cultivated, they must first be freed from the surrounding cells and mucus. This is achieved by dissolving the material in Sputofluol®. Sputofluol® contains alkaline hypochlorite which dissolves the organic material by oxidation without damaging the acid-alcohol-resistant tubercle bacilli. The undesired accompanying bacterial flora is destroyed.

Experimental procedure and evaluation

Place at least 4 ml sputum, urine, punctate, sediment etc. in a sterile centrifuge tube together with approximately 12 ml (ratio 1:3) of a 10–15% Sputofluol® solution prepared with sterile, distilled water (the strength of the solution depends on the degree of contamination), mix with a sterile glas rod. Allow to react for 10 min then centrifuge at 3000 to 4800 rpm for 20 min. The supernatant is decanted. For microscopical detection of bacilli, smear the sediment on an unused, fat-free slide, allow to dry, fix careful over a flame and then stain in the customary way. When identifying tubercle bacilli in culture, a 5–10% Sputofluol® solution is used. In order to prevent irreversible damage to the tubercle bacilli due to the prolonged action of Sputofluol®, after 10 min of activity the solution is immediatly neutralized. 1N HCl is added dropwise until an added pH indicator (e.g. neutral red) changes. Then centifugate. The sediment is inoculated onto appropriate culture media. In the examination of milk or tissue, sediments are obtained from 30 ml milk or 10–50 g tissue comminuted in sterile, physiological saline or sterile Ringer solution.



Culture, Tb stain

Ordering information

Product	Package size	Cat. No.
Sputofluol® for microbiology and microscopy	1 L	108000

Use of Sputofluol®: More clean sample material – mucus is destroyed, more easy to find mycobacteria with higher safety.

Mycobacteria staining acc. to Ziehl-Neelsen

The acid "fastness" of certain bacteria, e.g. Tb bacteria, results from a wax-like shell which, due to an acid reaction, prevents the germs releasing dyes which they have absorbed.

Solution

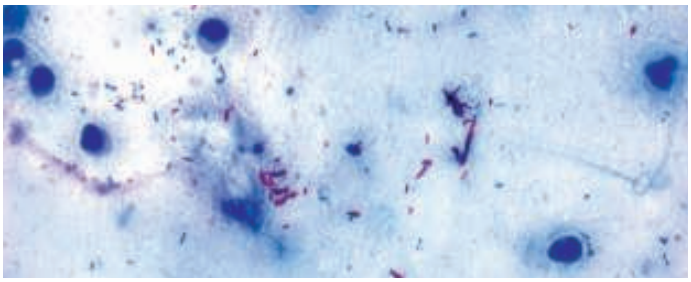
Methylene blue solution: Methylene blue 2 g; ethanol abs. 100 ml; dissolve at 40 to 50°C; filter after cooling; dilute 1+9 before use.

Experimental procedure and application

1) On the staining rack, cover fixed smears completely with Ziehl-Neelsen's carbol-fuchsin solution.
2) Heat three times carefully with a Bunsen burner from below until fumes form. Do not boil! Take your time and let the solution cool during the process. The dye should be allowed to work for 5 min in all.
3) Pour off the staining solution and rinse with a water jet.
4) Bleach with hydrochloric acid in ethanol until no further red clouds leave the normally thick parts of the preparation.
5) Rinse with water.
6) Restain for 1 min with diluted methylene blue solution.
7) Wash with water and dry in air.

Results

Acid-fast mycobacteria: red
Background: light blue



Culture, Tb-color modified (Ziehl-Neelsen method)

Diagnosis

A positive result means "acid-fast bacteria detected" and a negative result "acid-fast bacteria not detected". Is is impossible to say whether these bacteria are tubercle bacilli or other "atypical" mycobacteria or whether they are still capable of reproduction or are already dead. In sputum, positive in open lung Tb. Also Tb bacteria can be detected in urine, liquor and gastic juice with the Ziehl-Neelsen method. Apart from Mycobacterium tuberculosis, other acid-fast bacteria are the leprosy pathogen and many harmless saprophytes (e.g. smegma bacteria, Nocardia species).

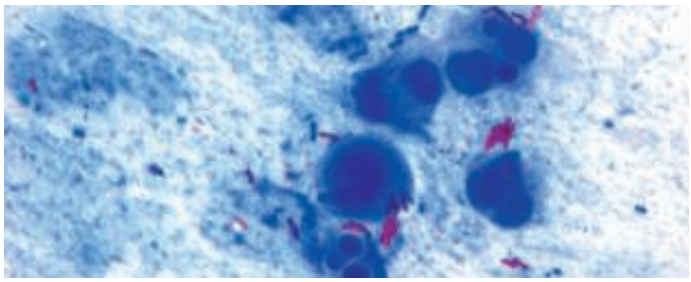
Ordering information

Product	Package size	Cat. No.
Ziehl-Neelsen carbol-fuchsin solution for microscopy	100 ml, 500 ml, 2.5 L	109215
Löffler's methylene blue solution for microscopy	100 ml, 500 ml, 2.5 L	101287
Hydrochloric acid in ethanol for microscopy ACS, ISO, Reag. Ph Eur	1 L, 5 L	100327
Methylene blue (C.I. 52015) for microscopy Certistain®	25 g, 100 g	115943
Ethanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100983
Lugol's solution (diluted iodine-potassium iodide solution) for the Gram staining method	250 ml, 1 L	109261

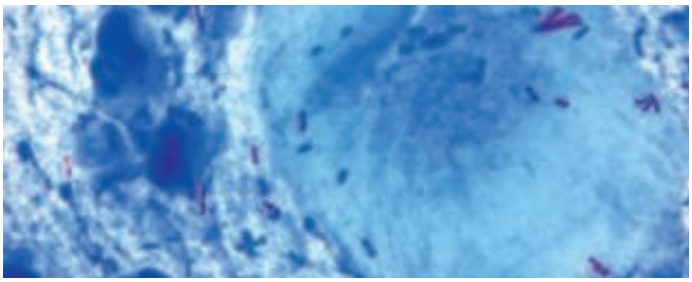
Convenience and full flexibility: Kits with all required reagents are available or single solutions for higher demands.

Tb-color modified, hot staining

Staining kit for the detection of mycobacteria (AFB) by hot staining method. Mycobacteria are difficult to stain because of the high proportion of lipid and wax in their cell walls. Up to now, in order to carry out the classical Ziehl-Neelsen staining, the test material to be heated with carbol-fuchsin solution to produce the mycolic acid fuchsin compound. Once stained, acid fast mycobacteria keep their coloring even after treatment with strong decolorizing solutions as HCl-ethanol. They remain red after counterstaining with methylene blue, whereas the microorganisms susceptible to acid take on the blue.



Culture, Tb-color modified



Culture, Tb-color modified

Application
The microscopic investigation of mycobacteria. Tb-color modified staining set uses the classical Ziehl-Neelsen hot staining procedure with methylene blue counterstraining.

Sample material
Heat-fixed smears of sputum, FNAB, lavages, imprints, body fluids, exsudates, puss, liquid, solid cultures and 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. One 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 to 4800 rpm 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.

Staining with a staining bench
1) Cover specimens completely with Ziehl-Neelsen's carbol-fuchsin solution. Carefully heat 3 times from below with Bunsen burner to steaming and keep hot for 5 min. Do not allow the stain to boil.
2) Wash with tap water until no further color is given off.
3) Cover completely with hydrochloric acid in ethanol solution and, depending on the thickness of the specimen, allow to stand for 15–30 sec.
4) Wash immediately with tap water.
5) Counterstain for 30 sec in Löffler's methylene blue solution or 1 min in diluted Löffler's methylene blue solution (dilution: 1+9 with dist. water).
6) Wash well with tap water.
7) Dry.

Dehydrate histological specimens (ascending alcohol series), clear in xylene or Neo-Clear® and mount with Entellan® new or Neo-Mount®.

Results

Acid-fast bacteria: red
Background: light blue

Diagnosis

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 these 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.

Capacity

The kit is sufficient for 250 specimen, stained on a staining rack.

Ordering information

Product	Package size	Cat. No.
Tb-color modified		100497
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 Löffler's 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	100 ml, 500 ml, 2.5 L	101287
Hydrochloric acid in ethanol	1 L, 5 L	100327
Acetone for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100014

Cold staining of Mycobacteria with Tb-color

Staining set for the microscopic examination of Mycobacteria. In the present modification of the Ziehl-Neelsen staining method, consistently good staining results are obtained without to heating the carbol-fuchsin solution (phenol vapours) during staining (cold staining).

Tb-color for the microscopic investigation of mycobacteria (cold staining)

Preparation with Sputofluol®

Add 3 parts of a 15% Sputofluol® solution prepared with distilled water to 1 part of the specimen (at least 4 ml) in a centrifuge tube. Allow to act for 10 min while shaking vigorously, centrifuge for 20 min at 3000 to 4800 rpm. Decant the supernatant and use the sediment to prepare a smear.

Fixation

Fix over a Bunsen burner flame (2–3 times taking care not to heat too strongly). Alternatively the smears can be fixed for 20 min at 100–110°C in a drying cabinet. Intense heat affects stainability.

Staining with the staining bench

1) Cover the air-dried, heat-fixed preparation completely with solution 2 (carbol-fuchsin solution) and stain for 5 min.
2) Wash off with tap water until clouds of stain cease to be formed.
3) Cover completely with solution 3 (decolorizing solution) and wash off immediately with tap water (maximum staining period 30 sec).
4) Completely cover the preparations with solution 4 (malachite green solution) and counterstain for 1 min.
5) Wash off for about 10 sec with tap water and dry in air.

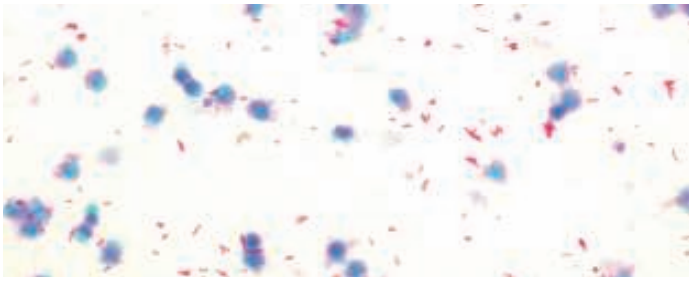
Staining in jars

1) Stain with carbol-fuchsin solution.	5 min
2) Wash in tap water.	15 sec
3) Treat with decolorizing solutione.	45 sec
4) Wash with tap water.	15 sec
5) Stain with malachite green solution.	1 min
6) Wash with tap water.	10 sec
7) Dry.	3 min

Examine the stained preparation under the microscope for at least 5 min using bright-field illumination and a 90–100x objective with immersion oil.

Staining results

Acid fast bacteria: red
Background: light green



Culture, Tb-color

Diagnosis

A positive result means "acid-fast bacteria detected" and a negative result "acid-fast bacteria not detected".
Is is impossible to say whether these bacteria are tubercle bacilli or other "atypical" mycobacteria or whether they are still capable of reproduction or are already dead.

Storage/shelf life

Do not store below +15°C as the stain then precipitates out. If stain precipitate is formed, place the bottle for 2 until 3 hours in water at a temperature of about 60°C. Filter prior to use. If stored as directed, the set is stable for at least 24 months.

Capacity

The kit is sufficient for 250 specimen, stained on the staining bench.

Ordering information

Product	Package size	Cat. No.
Tb-color staining kit		116450
Kit components		
Solution 1: Sputofluol®	500 ml	
Solution 2: Tb-color carbol-fuchsin solution	500 ml	
Solution 3: Hydrochloric acid in ethanol	500 ml	
Solution 4: Tb-color malachite green (oxalate) solution	500 ml	
Single reagents		
Tb-color carbol-fuchsin solution for the microscopic cold staining of mycobacteria	500 ml, 2.5 L	108512
Hydrochloric acid in ethanol for microscopy	1 L, 5 L	100327
Tb-color malachite green (oxalate) solution for the microscopic investigation of mycobacteria (cold staining)	500 ml	110630

Auramine staining of Mycobacteria acc. to Hagemann-Herrmann

Solutions

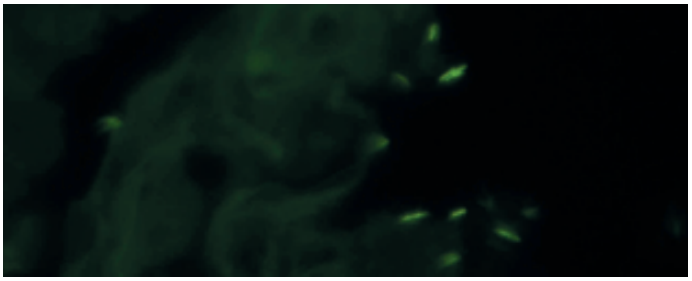
1. Phenol Auramine solution: Auramine 1 g; demineralized water 1 L; liquified phenol 50 ml.
2. Liquified phenol: Melt 10 parts of phenol in a slightly warmed water bath and add 1 part of water.
3. Potassium permanganate solution 0.1%: Potassium permanganate 1.0 g; demineralized water to 1 litre.

Experimental procedure and application

1) After heat fixing, bring the smears covered with phenol Auramine solution (1) to boil and stain for 5 min, shake off superfluous solution and repeat staining.
2) Rinse with water.
3) Differentiate in hydrochloric acid in ethanol until bleached (15–20 sec).
4) Rinse with water if necessary counterstain.
5) Dip for 5 sec into potassium permanganate solution (2).
6) Rinse with water.
7) Dip for 1 sec into Löffler's methylene blue solution.
8) Rinse with water.

Results

Tb bacteria: gold-yellow fluorescence
Cells and mucus: dark violet fluorescence



Culture, Auramine stain

Ordering information

Product	Package size	Cat. No.
Hydrochloric acid in ethanol for microscopy	1 L, 5 L	100327
Potassium permanganate for analysis EMSURE® ACS, Reag. Ph Eur	250 g	105082
Phenol GR for analysis ACS, Reag. Ph Eur	250 g, 1 kg	100206
Auramine O (C.I. 41000) for microscopy	50 g	101301
Löffler's methylene blue solution for microscopy	100 ml, 500 ml, 2.5 L	101287

Fluorescence staining of Mycobacteria with TB-fluor

The reason for the fact that the mycobacteria are acid-fast is that a wax-like coating (mycolic acid) in the membrane of these microorganisms prevents the release, on acid treatments, of the dye, once it has been absorbed. Both staining methods – Ziehl-Neelsen (with heated carbol-fuchsin) or Tb-color (cold method) for optical microscopy and TB-fluor (Auramine-Rhodamine) for fluorescence microscopy – are based on this principle.

Specimen for investigation

Sputum, pleural fluid, bronchio-alveolar washing (BAL), urine. The staining can also be performed in histology for acid-fast examination in lymphnodes and any other kind of biopsies.

Pretreatment of the specimen

Sputum

Before the staining, it is recommended to treat the sputum with Sputofluol® in order to free the mycobacteria of the coating of cells and mucus. One ingredient of Sputofluol® is alkaline hypochlorite. This agent dissolves the organic material by oxydation, while essentially leaving the acid-fast bacilli unaffected.
Three parts of a 15% solution of Sputofluol® are added to one part of specimen (4 ml) in a centrifuge tube. The mixture is shaken vigorously for 10 min. It is then centrifuged at 3000 to 4800 rpm for 20 min. The supernatant is decanted and the sediment is smeared thinly on degreased slides and dried in air.

Pleural fluids, bronchio-alveolar washing, urine

The specimens have to be smeared thinly on glass slides and dried in air after appropriate enrichment.

Histological specimens

The histological specimens will be treated to remove paraffin and rehydrate the section.

Fixation of smears

Fixation is carried out over the flame of a Bunsen burner (2 or 3 times rapidly, avoiding excessive heating). The smears can also be fixed for 20 min in an oven (100–110°C) or on a warming plate.

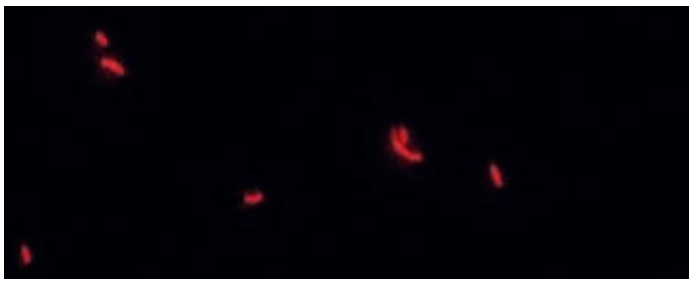
Staining in jars

1)	Stain with Auramine-Rhodamine solution.	15 min
2)	Rinse with tap water.	10 min
3)	Treat with decolorizing solution.	1 min
4)	Rinse with tap water.	5 min
5)	Counterstain with buffered $KMnO_4$ solution.	5 min
6)	Rinse with tap water.	5 min

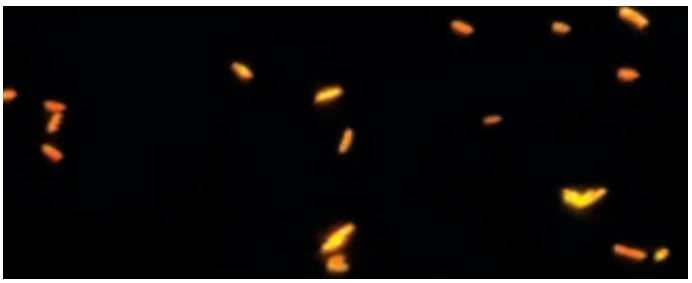
Dry the smears in air and mount them with Entellan® new; the histologigal sections should be treated with increased concentrations of alcohol and with xylene/Neo-Clear® before mounting with Entellan® new/Neo-Mount®.

Application for instruments

1)	Stain with Auramine-Rhodamine solution (Station 4).	15 min
2)	Rinse with tap water (Station 5).	10 min
3)	Treat with decolorizing solution (Station 3).	1 min
4)	Rinse with tap water (Station 5).	5 min
5)	Counterstain with buffered $KMnO_4$ solution (Station 2).	5 min
6)	Rinse with tap water (Station 5).	5 min
7)	Dry (Station 6).	5 min



Culture, TB-fluor, Rhodamine



Culture, TB-fluor, Auramine

Staining result and assessment

The acid-fast bacilli are clearly distinguished by being red-orange or green (depending of the filter combination used for the fluorescence microscope) on a dark background (25 x or 40 x objective).

Recommended filter combination

Exciation filter: 490–570 nm
Dichromatic mirror: 525 and 635 nm
Suppression filter: 505–600 nm

Diagnosis

A positive result means "acid-fast bacteria detected" and a negative result "acid-fast bacteria not detected".
Is is impossible to say whether these bacteria are tubercle bacilli or other "atypical" mycobacteria or whether they are still capable of reproduction or are already dead. Confirmation tests (culture, PCR, or similar methods) are requested in order to establish the diagnosis of tuberculosis.

Double staining

Any doubtful or suspicious result can be confirmed by conducting the double staining method "TB-fluor – Tb-color" or "TB-fluor – Tb-color modified". In the case of the unmounted 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 a light green (Tb-color) or light blue (Tb-color mod.) background.

Storage

Storage temperature between 15°C and 25°C. Unopened, the staining kit has a shelf life of 24 months.

Stability

In case of manual staining in jar or with a staining instrument, the staining kit will allow to stain 300 up to 400 specimens, depending of the number of slides placed in the jar for each staining procedure. It is recommended to replace the solution 1 (Auramine-Rhodamine) after 10 to 15 staining series and 3 (Counterstaining solution) after 5 to 10 staining series; solution 2 (Decolorizing solution) should be replaced after 5 staining series.
If stored below +15°C, a precipitate of dye may form in solution 1 and 3. If this occurs, place the bottle in water at about 60°C for 2–3 hours. Filter before use.

Ordering information

Product	Package size	Cat. No.
TB-fluor		109093
Kit components		
Solution 1: Auramine-Rhodamine solution	500 ml	
Solution 2: Decolorizing solution (HCL-Isopropanol)	3 x 500 ml	
Solution 3: Counterstaining solution (Buffered $KMnO_4$)	2 x 500 ml	

TB-fluor phenol-free

Fluorescence staining kit for the microscopic investigation of mycobacteria. The acid-fastness of mycobacteria is based on the fact that a wax-like sheath of the membrane of these bacteria prevents the release of already incorporated dyes by treatment with acid. Here the use of a modified staining solution makes the inclusion of phenol in the staining solution unnecessary. The sensitivity and specifity of the staining results are identical with those obtained using the classical (phenol-employing) staining method.

Examination material

Specimen materials offering themselves for examination with this method include e.g. sputum, specimens gained by pleural punctation or bronchial lavage (BAL), urine sediments, FNAB, imprints, culture specimens, and histologic sections.

Sample preparation

The sample material is applied to clean, degreased microscopic slides.

Sputum

Sputum should be pretreated with Sputofluol® in order to free the mycobacteria from the enveloping mucus. One 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 to 4800 rpm for 20 min, decant the supernatant, smear out the sediment, and allow to dry.

Punctation and lavage material, sediments

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

Histologic sections

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

Fixation

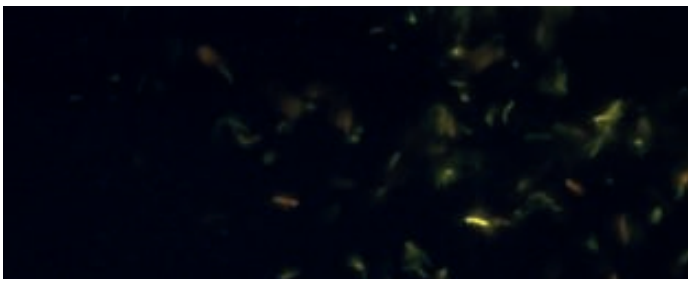
Fixate samples over the bunsen burner flame (2–3 times, taking care to avoid excessive heating).
The material may also be fixated at 100–110°C for 20 min in a drying cabinet or on a hotplate.

Staining on the staining bench

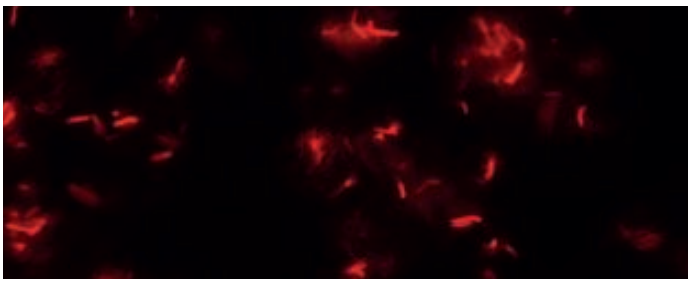
1)	Cover the air-dried, heat-fixated specimens completely with Auramine-Rhodamine staining solution and stain.	15 min
2)	Carefully rinse under running tap water.	30 sec
3)	Cover the specimens completely with decolorizing solution and leave to stand.	1 min
4)	Carefully rinse under running tap water.	30 sec
5)	Cover the specimens completely with $KMnO_4$ counterstaining solution and stain.	5 min
6)	Carefully rinse under running tap water.	30 sec

Allow the specimens to dry and, if necessary, mount with Entellan® new or Neo-Mount®. Dehydrate histologic specimens (ascending alcohol series) and mount with Entellan® new or Neo-Mount®.

Note: Carefully shake the Auramine-Rhodamine solution prior to use.



Lung section, TB-fluor phenol-free, Auramine stain



Lung section, TB-fluor phenol-free, Rhodamine stain

Staining results

Acid-fast bacteria: red TB-fluor or yellow-green fluorescence
Background: dark

Recommended filter combination

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

Double staining

Any doubtful or suspicious result can be confirmed by conducting the double staining method "TB-fluor – Tb-color" or "TB-fluor – Tb-color modified". In the case of the unmounted 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 a light green (Tb-color) or light blue (Tb-color mod.) background.

Capacity

The kit is sufficient for 60–65 specimen.

Ordering information

Product	Package size	Cat. No.
TB-fluor phenol-free		101597
Kit components		
Solution 1: Auramine-Rhodamine solution, phenol-free	200 ml	
Solution 2: Decolorizing solution (HCL-Isopropanol)	2 x 200 ml	
Solution 3: Counterstaining solution (Buffered $KMnO_4$)	200 ml	

TB fluorescence staining without phenol:
Same specifity and sensitivity with less hazardous reagents.
Phenol is substituted in the staining solution!

Brucella staining acc. to Koslowskij-Treffenstädt

Solutions

1. Safranine solution 3%: Safranine 3 g; demineralized water to 100 ml; dissolve under heat and filter.
2. Acetic acid 1%: Glacial acetic acid 1 ml; demineralized water to 100 ml.
3. Methylene blue solution: Methylene blue 1 g; demineralized water to 100 ml.

Experimental procedure and application

1) After heat fixing (or methanol fixing), cover smear with safranine solution (1), heat for 3 min until it bubbles.
2) Rinse thoroughly with water. With thick smears, differentiate with acetic acid (2).
3) Counterstain with methylene blue solution (3).
4) Rinse.

Results

Brucella: red
Cells, other bacteria: blue

Ordering information

Product	Package size	Cat. No.
Safranine O (C.I. 50240) for microscopy Certistain®	25 g	115948
Methylene blue (C.I. 52015) for microscopy Certistain®	25 g, 100 g	115943
Methanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	106009
Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100063

Capsule staining in Pneumococci

Solutions

1. Fuchsin solution: New fuchsin 2.0 g; ethanol abs. 100 ml.
2. Acetic acid 3%: Glacial acetic acid 3 ml; demineralized water to 100 ml.

Experimental procedure and application

Place exsudate or sputum on the slide with a loop and spread. Dry quickly by waving in air and on the staining rack pour 1 drop of ethanol on the smear. Ignite this immediately and extinguish after 1 sec.
The re-ignite and re-extinguish until no alcohol remains. After cooling, briefly stain with acetic acid (2) and restain with alcoholic fuchsin solution (1).

Result

Pneumococci: dark red in a pale pink zone

Ordering information

Product	Package size	Cat. No.
New fuchsin (C.I. 42520) for microscopy Certistain®	100 g	105226
Ethanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100983
Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100063

Capsule staining acc. to Olt in anthrax pathogens

Solution
Safranine solution 3%: Safranine 3.0 g; demineralized water to 100 ml; dissolve under heat and filter.

Experimental procedure and application
After heat fixing, stain with safranine solution for 1–2 min, rinse with water and dry. Prior to the examination cover the smear with water and a cover glass.

Result
Capsules of anthrax pathogens: orange

Ordering information

Product	Package size	Cat. No.
Safranine O (C.I. 50240) for microscopy Certistain®	25 g	115948

Negative visualisation of capsules

Solution
Nigrosine solution: Nigrosine 5 g; demineralized water 100 ml; boil for 10 min and allow to cool; add formaldehyde solution 0.5 ml; filter.

Experimental procedure and application
Rub 1 loop of nigrosine solution on a pre-cleaned slide together with some bacteria material. Press a clean cover glass over the slide.

Results
Capsules: bright
Background: dark

Ordering information

Product	Package size	Cat. No.
Nigrosine (C.I. 50420) water-soluble for microscopy Certistain®	25 g	115924
Formaldehyde solution min. 37% free from acid stabilized with about 10% methanol and calcium carbonate for histology	1 L, 2.5 L	103999

Cilium staining acc. to Lembach and Sous

Solutions

1. Tannin solution: Tannic acid 20.0 g; demineralized water to 100 ml.
2. Chromic acid solution: Chromium (VI) oxide 2.5 g; demineralized water to 100 ml.
3. Silver nitrate solution: Silver nitrate 25 g; demineralized water to 100 ml.
4. Sodium sulfate solution: Sodium sulfate decahydrate 60 g; demineralized water to 100 ml.
5. Ammonia solution 1%: Ammonia solution 25% 4.4 ml; demineralized water to 100 ml.
6. Mordant: Tannin solution (1) 100 ml; chromic acid solution (2) 15 ml; keep for two days at room temperature; filter before use.
7. Standard solution: Silver nitrate solution (3) 20 ml; sodium sulfate solution (4) 100 ml; pour off supernatant, wash the precipitate out thoroughly with demineralized water and take-up in 500 ml of demineralized water. In a dark bottle it can be kept for long periods.
8. Working solution: Drop by drop add ammonia solution (5) to approx. 25 ml of standard solution until the brown precipitate disappears. Make up the solution fresh each time before use.

Experimental procedure and application

1) Put three drops of tap water onto a clean, fat-free slide. Place the culture carefully in the first drop, after 1 min transfer it to the second drop and after a further minute to the third drop. From this, take a loopful and place it on a slide or cover glass. Dry in air and fix (once through the flame).
2) Cover the slide or cover glass with mordant (6) and allow to act for 30 min.
3) Then pour on the working solution (8) and heat gently until a pale brown sheen appears on the specimen.
4) Rinse with water.
5) Restain with Gram's crystal violet solution for 3 min.
6) Rinse with water and dry high above the flame.

Results

Cilia: grey-black
Bacteria bodies: blue-violet

Note

The colored visualisation of cilia is most successful with material from swarm plates.

Ordering information

Product	Package size	Cat. No.
Tannic acid powder suitable for use as excipient EMPROVE® exp Ph Eur, USP	1 kg	100773
Chromium(VI) oxide GR for analysis EMSURE®	250 g	100229
Silver nitrate for analysis EMSURE® ISO, Reag. Ph Eur	25 g, 100 g	101512
Sodium sulfate decahydrate for analysis EMSURE® ISO, Reag. Ph Eur	1 kg	106648
Ammonia solution 25% for analysis EMSURE®	1 L, 2.5 L	105432
Gram's crystal violet solution for the Gram staining method	500 ml, 2.5 L	109218

Spore staining acc. to Rakette

Solutions

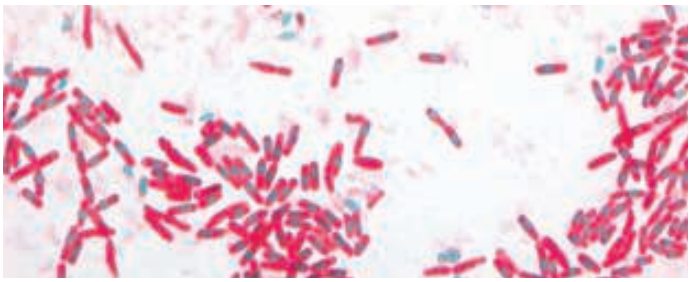
1. Malachite green solution: Malachite green 5.0 g; demineralized water to 100 ml.
2. Eosin solution: Eosin Y 2.5 g; demineralized water to 100 ml.
3. Safranine solution: Safranine 0.5 g; demineralized water to 100 ml.

Experimental procedure and application

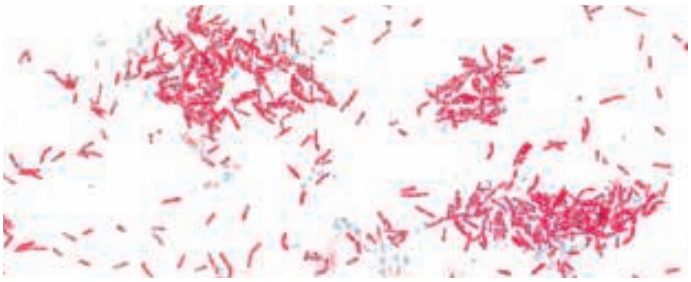
1) Fix the air dried smear: draw 6 to 8 times through the flame.
2) Staining: cover the slide completely with malachite green solution (1), bring to boil for 20 sec and allow to act for 30 sec, if necessary somewhat longer.
3) Rinse for 30 sec in running water.
4) Restaining: 1 min with eosin solution (2) or, acc. to Wirtz, 30 sec with safranine solution (3).
5) Rinse and dry.

Results

Spores: Emerald green
Other cell parts: red



Culture, Rakette stain



Culture, Rakette stain

Ordering information

Product	Package size	Cat. No.
Malachite green oxalate (C.I. 42000) for microscopy Certistain®	25 g, 100 g	115942
Eosin Y (yellowish) (C.I. 45380) for microscopy Certistain®	25 g, 100 g	115935
Safranine O (C.I. 50240) for microscopy Certistain®	25 g	115948

Spirochaeta staining with Giemsa solution

Solutions

1. Diluted Giemsa solution: Giemsa's azure eosin methylene-blue solution 10 drops; demineralized water 20 ml.
2. Potassium carbonate solution: Potassium carbonate 0.1 g; demineralized water to 100 ml.

Experimental procedure and application

1) Fixing with methanol.
2) Allow the diluted Giemsa solution (1) to work for 12–24 hours or, after adding a few drops of potassium carbonate solution (2), for 6–8 hours. To avoid the formation of precipitate, lay the specimen on the lower side of the glass rod in the staining solution.
3) Rinse with water.

Results

Spirochaeta pallida: pink
Other Spirochaeta: reddish to bluish-violet

Ordering information

Product	Package size	Cat. No.
Giemsa's azure eosin methylene blue solution for microscopy	100 ml, 500 ml, 1 L, 2.5 L	109204
Methanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	106009
Potassium carbonate for analysis EMSURE® ACS, ISO, Reag. Ph Eur	500 g	104928

Trichomonads staining with Cytocolor®

The Cytocolor® staining set is for the differential coloring of smear preparations in cytodiagnosis using the standard stain acc. to Szczepanik (modified Papanicolaou staining). With this method Trichomonas vaginalis can be readily recognized.

Experimental procedure and application

The smears, fixed with Merckofix®, are placed one after the other in the following solutions:

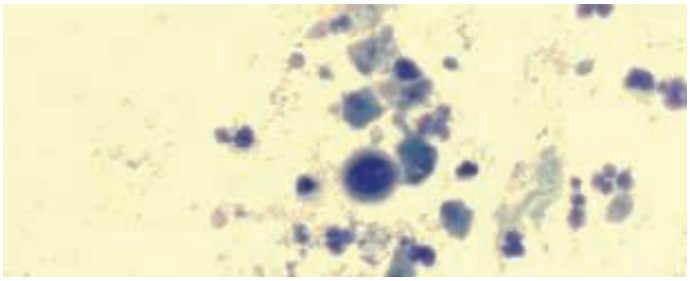
1)	Distilled water	10 x 1 sec
2)	Modified hamatoxylin solution	1 x 1 min
3)	Wash under running water	1 x 5 sec
4)	2-Propanol for analysis	2 x 1 sec
5)	Modified polychrome solution	1 x 1 min
6)	80% 2-Propanol	5 x 1 sec
7)	2-Propanol	5 x 1 sec
8)	2-Propanol	5 x 1 sec
9)	Xylene	5 x 1 sec
10)	Xylene	5 x 1 sec

The stained smears can be covered with Merckoglas® or with cover glass and Entellan® new.

Result

Trichomonads: grey-blue to grey-green

Gynecological smear, Cytocolor®



Gynecological smear, Cytocolor®

Ordering information

Product	Package size	Cat. No.
Cytological standard stain acc. to Szczepanik for microscopy Cytocolor®		115355
Kit components		
Solution 1: Modified hematoxylin solution	500 ml	
Solution 2: Modified polychrome solution	500 ml	
Solution 3: 2-Propanol	3 x 500 ml	
Solution 4: Xylene	500 ml	
Merckofix® spray fixative for cytodiagnosis	100 ml	103981
Entellan® new rapid mounting medium for microscopy	100 ml, 500 ml, 1 L	107961
Merckoglas® liquid cover glass for microscopy	500 ml	103973

Fungi visualisation in the original preparation

Mode of action

Strong alkalines cause the examination material to well which makes the refractive fungi elements more clearly visible. This process can be enhanced by gentle heating.

Experimental procedure and evaluation

Place 1 drop of alkaline (10–30%) and examination material (from the edges of the skin changes) on the slide. Warm carefully. Allow the alkaline to work for 5 to 15 min until a gelatinous consistency is obtained. Squash the examination material with a cover glass, and with a switched down drying system, observe the material under the microscope.

Note

It is only necessary to use oil immersion to observe actinomycetes and streptomycetes. If heating is too intensive the alkaline crystallizes out.

Ordering information

Product	Package size	Cat. No.
Sodium hydroxide solution min. 10% (1.11) for analysis EMSURE®	1 L	105588
Sodium hydroxide pellets for analysis EMSURE® ISO	500 g	106498
Potassium hydroxide pellets for analysis EMSURE®	500 g	105033
Tetramethylammonium hydroxide solution 10% for polarography and for examination of steroids Reag. Ph Eur	50 ml	108123

Fungi staining with Lactophenol blue

Experimental procedure and application

1) Clear the specimen for 1 to 15 min, depending on its thickness, with 1 to 2 drops of alkaline.
2) Apply several changes of water and soak up with filter paper.
3) Stain with 1 to 2 drops of lactophenol blue solution and cover about 2 min.

Note

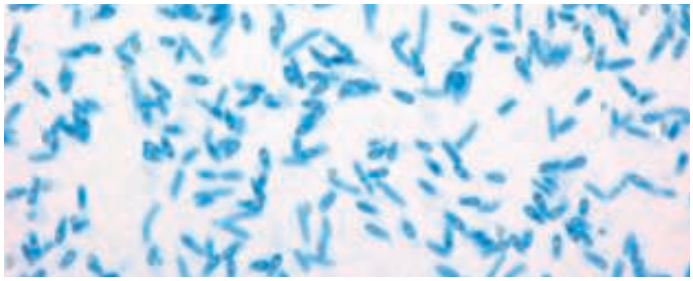
For untreated culture specimens, apply 1 to 2 drops of lactophenol blue solution and cover with a cover slip. Examine under the microscope after about 2 min.

Result

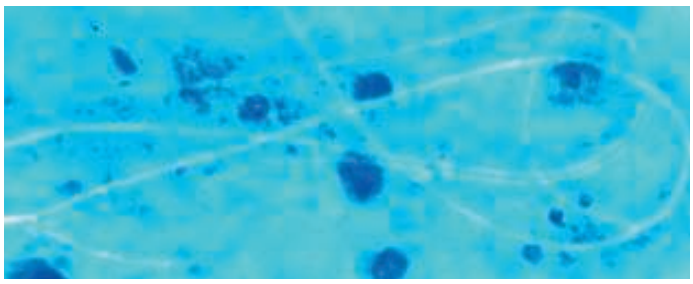
Fungal elements: dark blue

Ordering information

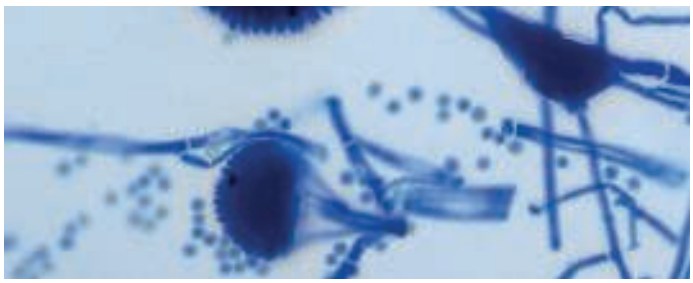
Product	Package size	Cat. No.
Lactophenol blue solution for staining fungi	100 ml	113741
Sodium hydroxide pellets for analysis EMSURE® ISO	500 g	106498
Potassium hydroxide pellets for analysis EMSURE®	500 g	105033



Culture, Lactophenol blue stain



Culture, Lactophenol blue stain



Culture, Lactophenol blue stain

Methenamine silver plating kit acc. to Gomori

Argentaffin structures as fungi can be isolated well by methenamine silver plating, the target structures appear in dark brown to black.

Material

3–5 µm thick paraffin sections

Preparation

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.

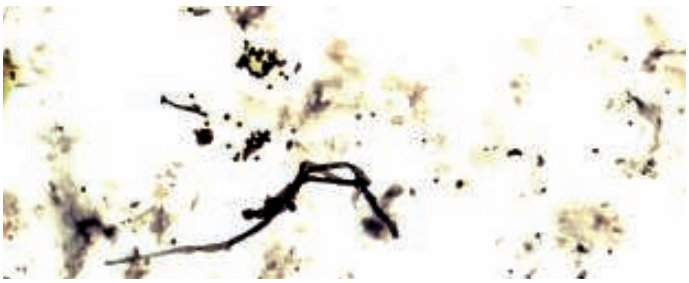
Procedure

Deparaffinate and rehydrate sections in the usual way.	
Distilled water	2 min
Periodic acid solution	10 min
Distilled water	3 x ca. 30 sec
Stain in the water bath at 52°–57°C	
with silver nitrate/methenamine borate solution	35–45 min
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, clear and mount the sections	

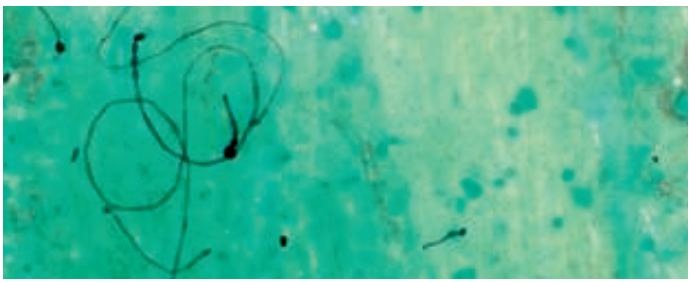
Mount the xylene–wet slides with Entellan® new or the Neo-Clear® wet slides with Neo-Mount® and cover glass.

Results

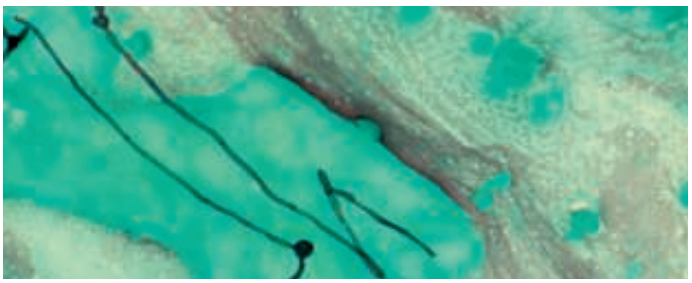
Fungi: dark brown to black
Membranes: dark brown to black
Background: green



Culture, Methenamine silver plating acc. to Gomori



Culture, Methenamine silver plating acc. to Gomori



Culture, Methenamine silver plating acc. to Gomori

Ordering information

Product	Package size	Ord. No.
Methenamine silver plating kit acc. to Gomori		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	

PAS-Fungal staining

If fungal material is kept for a long time in alkalines, the walls of the fungi are damaged. Therefore fine scales should not be treated with potassium or sodium hydroxide. For softening skin scrapings, nails or hair, use the preparation method acc. to Taschdjian (1955) and Muskat-Blit (1953). Any appropriate staining method can then follow.

Mode of action

Polysaccharides in the cell wall are converted by periodic acid into polyaldehyde which reacts with colorless Schiff's reagent (fuchsin sulphurous acid) forming a red-blue dye (PAS-reaction = Periodic-Acid-Schiff-reaction). The coloring shows the fungal elements clearly.

Solutions

1. Lactic acid solution 10%: Lactic acid 90% 11 ml; demineralized water 88 ml.
2. Periodic acid solution: Periodic acid 1.0 g; demineralized water 20 ml; always make up fresh before use.
3. Thionine solution: Thionine 1.0 g; demineralized water to 50 ml.

Preparing the specimen

1) Depending on the thickness of the examination material, allow a 10% alkaline solution to act for 5 to 15 min until decoloration and softening occurs. Do not let the material become too "soupy".
2) Soak up the superfluous alkaline with filter paper.
3) Cover the specimen with lactic acid solution (1) and let it act for about 3 min; test with pH indicator if necessary. The pH should be between 3 and 5.
4) Soak up superfluous acid.
5) Add a few drops of 96% ethanol to the material on the slide and allow it to dry for several hours. If the material does not stick to the slide, place it on a slide covered with albumin or on a piece of adhesive tape. After this staining can take place.

Staining

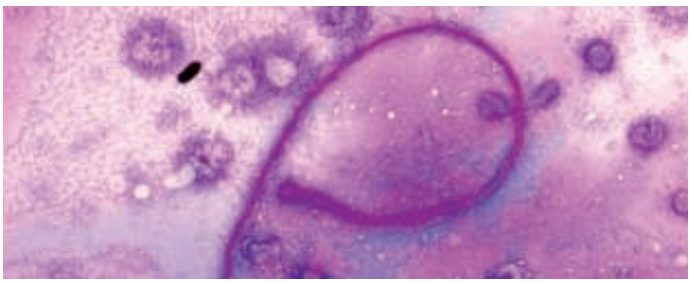
1) Allow 5% periodic acid solution (2) to act for 10 min.
2) Rinse for 2 min under running tap water.
3) Stain for 15 min with Schiff's reagent.
4) Removes superfluous Schiff's reagent by applying 2% thionine solution (3) for 10 min.
5) Rinse under running tap water.

Notes

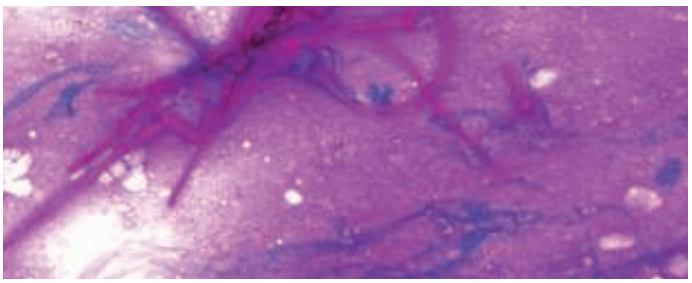
1) With skin scrapings, thionine application is not necessary. This shortens the staining time.
2) Small skin particles can be stuck to adhesive film and placed in the staining dishes. For preparations which are to kept, cut out part of the film strip and embed this in Kaiser's glycerol gelatin. Because the adhesive film is attacked by xylene and alcohol, canada balsam and dehydration in an increasing alcohol series can not be use.
3) Coarse tissue particles on the adhesive film are softened before PAS staining to a semi-gelatinous consistency.

Result

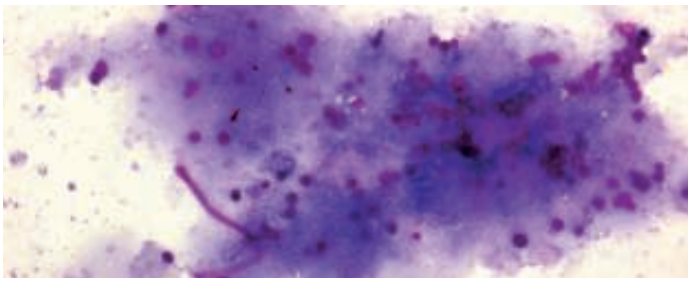
Fungal elements: red-blue



Culture, PAS stain



Culture, PAS stain



Culture, PAS stain

Culture, PAS stain

Ordering information

Product	Package size	Cat. No.
Sodium hydroxide solution min. 10% (1.11) for analysis EMSURE®	1 L	105588
Sodium hydroxide pellets for analysis EMSURE® ISO	500 g	106498
Potassium hydroxide pellets for analysis EMSURE®	500 g	105033
Tetramethylammonium hydroxide solution 10% for polarography and for examination of steroids Reag. Ph Eur	50 ml	108123
pH-indicator strips pH 0 – 6.0 non-bleeding pH 0 – 0.5 – 1.0 – 1.5 – 2.0 – 2.5 – 3.0 – 3.5 – 4.0 – 4.5 – 5.0 – 5.5 – 6.0 Acilit®	100 strips	109531
(S)-Lactic acid about 90% suitable for use as excipient EMPROVE® exp Ph Eur, BP, E 270	2.5 L	100366
Ethanol absolute for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100983
Periodic acid for analysis EMSURE®	25 g, 100 g	100524
Schiff's reagent for microscopy	500 ml, 2.5 L	109033
Thionine (acetate) (C.I. 52000) for microscopy Certistain®	25 g	115929
Albumin fraction V (from bovine serum) for biochemistry	25 g, 100 g	112018
Kaiser's glycerol gelatine for microscopy	100 g	109242

Oil immersion

Immersion media for microscopy have nearly identical refractive indices as glass. Immersion oils practically eliminate light beam deflection so that the effectiveness of the lens is considerably increased.

Application

When microscoping, first locate the part of the dry specimen to be investigated. Swing the lens holder away, place a drop of immersion oil on the specimen at the point to be observed and return the lens to their original position. When finished, clean the lens and the specimen with ethanol.

Ordering information

Product	Package size	Cat. No.
Immersion oil for microscopy	100 ml, 500 ml	104699
Immersion oil acc. to ISO 8036 modified for microscopy	100 ml	115577
Oil of cedar wood for microscopy	100 ml, 500 ml	106965

Immersion oil: The refractic index is around 1.5 and the difference for the convenience of the application is based on the different viscosities.

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