1.15937.0025
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Microscopy

Fuchsin (C.I. 42510)

for microscopy Certistain®

For professional use only

IVD In Vitro Diagnostic Medical Device CE

Intended purpose

for staining of acid-fast bacteria (AFB) according to Kinyoun

This staining dye "Fuchsin (C.I. 42510) - for microscopy Certistain®" is used for human-medical cell diagnosis and serves the purpose of the bacteriological and histological investigation of sample material of human origin. It is a dry staining dye that is used to prepare a staining solution, that when used together with other in vitro diagnostic products from our portfolio makes target structures (acid-fast bacteria (AFB)) evaluable for diagnostic purposes by fixing, embedding where necessary, staining with the above fuchsin solution, counterstaining, mounting in bacteriological and histological specimen materials, for example smears of enriched bacterial cultures or histological sections of e.g. the lung.

Unstained structures are relatively low in contrast and are extremely difficult to distinguish under the light microscope. The images created using the staining solutions help the authorized and qualified investigator to better define the form and structure in such cases. Further tests must be carried out according to recognized, valid methods to reach a definitive diagnosis.

Principle

The cell wall of acid-fast bacteria has a high proportion of wax and lipids and hence absorbs dyes only very slowly. As a measure to enhance the absorption of the fuchsin dye and thus the formation of the mycolate-fuchsin complex in the cell wall, the carbolfuchsin solution applied to the specimen is normally heated to evaporation point. If the Kinyoun carbolfuchsin solution is used, the heating of the solution is dispensable. Consequently, the release of hazardous phenolic vapors will be avoided.
Once the acid-fast bacteria have absorbed the fuchsin dye, it is virtually impossible to decolorize them again, even when they are intensively treated with a decolorizing solution such as e.g. hydrochloric acid in ethanol. Accordingly, acid-fast bacteria are termed as acid- and alcohol-fast for staining, and are stained red in the microscopic visualization. Correspondingly, all non-acid-fast microorganisms are counterstained with an appropriate dye. In the present operating instructions is counterstained accordance with methylene blue.
Pretreatment of the specimens with Sputofluol® dissolves the bacteria from the surrounding viscid sputum and cell material.

Sample material

Smears of bacteriological material that have been air-dried, heat-fixed, and pretreated with Sputofluol® like sputum, smears from fine needle aspiration biopsies (FNAB), rinses, imprints, effusions, pus, exsudates, liquid and solid cultures

Sections of formalin-fixed tissue of human origin, embedded in paraffin (3 - 4 µm thick paraffin sections)

Reagents

Cat. No. 115937
Fuchsin (C.I. 42510) 25 g, 100 g, 1 kg
for microscopy Certistain®
Color Index No.: 42510
Color Index Name: Basic violet 14, Solvent red 41

Also required:

Cat. No. 100206	Phenol GR for analysis ACS,Reag. Ph Eur	250 g, 1 kg
Cat. No. 100327	Hydrochloric acid in ethanol for microscopy	1 l, 5 l
Cat. No. 100974	Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 l, 2.5 l
Cat. No. 115943	Methylene blue (C.I. 52015) for microscopy Certistain®	25 g, 100 g

Alternatively:

Instead of the combination of single reagents, the staining kits 1.16450.0001 and 1.00497.0001 can be used:

Cat. No. 1.16450.0001
AFB-Color staining kit 1 set
for the microscopic investigation of acid-fast bacteria (AFB) (cold staining)

Cat. No. 1.00497.0001
AFB-Color modified 1 set
Staining kit for the detection of acid-fast bacteria (AFB) by hot staining method

Sample preparation

The sampling must be performed by qualified personnel.

All samples must be treated using state-of-the-art technology.
All samples must be clearly labeled.
Suitable instruments must be used for taking samples and their preparation.
Follow the manufacturer's instructions for application / use.

When using the corresponding auxiliary reagents, the corresponding instructions for use must be observed.

Sputum

The acid-fast bacteria should be pretreated with Sputofluol® to dissolve them from mucus and cellular structures. In this process, the active ingredient hypochlorite dissolves the organic material by oxidation and gently releases the acid-fast bacteria so that they can be processed further.

Reagent preparation: Preparation of Sputofluol® solution 15 %

For preparation of approx. 100 ml solution mix:

Sputofluol®	15 ml
Distilled water	85 ml

Preparing sample material in centrifuge tubes:	
Sample	1 part (min. 2 ml)
Sputofluol® solution (15 % in distilled water)	3 parts
Shake vigorously	10 min
Centrifuge at 3000 - 4800 rpm	20 min
Decant supernatant Prepare smears of the sediment Air-dry	

Punctates, lavages, sediments

After appropriate enrichment measures, smear sample material on the slide and allow to air-dry.

Histological sections

Deparaffinize sections in the conventional manner and rehydrate in a descending alcohol series. Pretreatment with Sputofluol® is not necessary for specimens fixed with formalin.

Fixation

Specimens are fixed over a Bunsen burner flame (2 - 3 times, taking care to avoid excessive heating).
The specimens can also be fixed by heating at 100 - 110 °C in a drying cabinet or on a heating plate for 20 min.
Excessive temperatures or prolonged heating may involve a deterioration of the staining performance.

Reagent preparation

Liquid phenol

Melt 10 parts phenol by warming and add 1 part distilled water.

Kinyoun solution

For preparation of approx. 1250 ml solution mix:

Fuchsin (C.I. 42510) Certistain®	40 g
Liquid phenol	80 ml
dissolve	
Ethanol 96 %	200 ml
Distilled water	1000 ml
add and mix	

Methylene blue solution 1 %

For preparation of approx. 1000 ml solution mix:

Methylene blue (C.I.52015) Certistain®	10 g
Distilled water	1000 ml
dissolve	

Methylene blue solution 0.1 %, working solution

For preparation of approx. 100 ml solution mix:

Methylene blue solution 1 %	10 ml
Distilled water	90 ml
mix	

The freshly prepared staining solutions should be filtered before use.

Smear samples

Procedure

Staining on the staining rack

The stated times should be adhered to in order to guarantee an optimal staining result.

Slide with fixed smear		
Kinyoun solution	cover completely and stain	5 min
Running tap water	rinse until no further clouds of dye are produced	
Hydrochloric acid in ethanol	cover completely and leave to react	15 - 30 sec*
Running tap water	rinse immediately	
Methylene blue solution 0.1 %, working solution	counterstaining	1 min
Running tap water	rinse carefully	
Air-dry (e. g. over night or at 50 °C in the drying cabinet)		

* depending on thickness of specimen

After dehydration (ascending alcohol series) and clarification with xylene or Neo-Clear®, bacteriological slides can be covered with non-aqueous mounting agents (e.g. Neo-Mount®, Entellan® new, or DPX new) and a cover glass and can then be stored. When left unmounted, the stain remains stable for approx. 3 days, covered with immersion oil for just a few hours.

The use of immersion oil is recommended for the analysis of stained slides with a microscopic magnification >40x.

Result

Acid-fast bacteria (AFB)	red
Background	light blue

Evaluation

A positive result means "acid-fast bacteria detected" and a negative result "acid-fast bacteria not detected". A positive result does not mean that a taxonomic classification by microscopy is possible. If acid-fast bacteria are detected, further analyses must be performed in specially equipped laboratories.
The vitality (active, inactive) of the bacteria can also not be determined.

Trouble-shooting

Fixation of smear samples

A sufficient degree of heat-fixing using a Bunsen burner or in a heating cabinet is essential to prevent the infectious potential of the specimens and further proliferation of the bacteria.
Excessive temperatures or prolonged heating may involve a deterioration of the staining performance.

No staining of acid-fast bacteria

The critical step of this staining process is the decolorizing step, which can be influenced by the thickness of the specimen smear.
In addition, a fresh solution of hydrochloric acid in ethanol is highly reactive, meaning that the result should be evaluated with caution. The incubation times stated in this protocol should be kept accurately in the decolorizing step, since otherwise false-negative results may ensue.

Technical notes

The microscope used should meet the requirements of a medical diagnostic laboratory.
The freshly prepared staining solutions should be filtered before use.

Remove surplus immersion oil before filing.

Diagnostics

Diagnoses are to be made only by authorized and qualified personnel.
Valid nomenclatures must be used.
This method can be supplementarily used in human diagnostics.
Further tests must be selected and implemented according to recognized methods.

Suitable controls (e.g. ISOSLIDE® AFB, Cat. No. 1.02560.0001) should be conducted with each application in order to avoid an incorrect result.

Storage

Store Fuchsin (C.I. 42510) - for microscopy Certistain® at +5 °C to +30 °C.

Shelf-life

Fuchsin (C.I. 42510) - for microscopy Certistain® can be used until the stated expiry date.

After first opening of the bottle, the contents can be used up to the stated expiry date when stored at +5 °C to +30 °C.

The bottles must be kept tightly closed at all times.

Additional instructions

For professional use only.
In order to avoid errors, the application must be carried out by qualified personnel only.
National guidelines for work safety and quality assurance must be followed.
Microscopes equipped according to the standard must be used.
If necessary use a standard centrifuge suitable for medical diagnostic laboratory.

Protection against infection

Effective measures must be taken to protect against infection in line with laboratory guidelines.

Instructions for disposal

The package must be disposed of in accordance with the current disposal guidelines.
Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines. Information on disposal can be obtained under the Quick Link "Hints for Disposal of Microscopy Products" at www.microscopy-products.com. Within the EU the currently applicable REGULATION (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 applies.

Auxiliary reagents

Cat. No.	100206	Phenol GR for analysis ACS,Reag. Ph Eur	250 g, 1 kg
Cat. No.	100327	Hydrochloric acid in ethanol for microscopy	1 l, 5 l
Cat. No.	100497	AFB-Color modified Staining kit for the detection of acid-fast bacteria (AFB) by hot staining method	1 set
Cat. No.	100579	DPX new non-aqueous mounting medium for microscopy	500 ml
Cat. No.	100974	Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 l, 2.5 l
Cat. No.	102560	ISOSLIDE® AFB Control Slides with reference tissue for the detection of acid-fast bacteria in histological tissue	25 tests
Cat. No.	103699	Immersion oil Type N acc. to ISO 8036 for microscopy	100-ml dropping bottle
Cat. No.	104699	Immersion oil for microscopy	100-ml dropping bottle, 100 ml, 500 ml
Cat. No.	107961	Entellan® new rapid mounting medium for microscopy	100 ml, 500 ml, 1 l
Cat. No.	108000	Sputofluol® for microscopy	1 l
Cat. No.	108298	Xylene (isomeric mixture) for histology	4 l
Cat. No.	108562	Aquatex® (aqueous mounting agent) for microscopy	50-ml dropping bottle
Cat. No.	109016	Neo-Mount® anhydrous mounting medium for microscopy	100-ml dropping bottle, 500 ml
Cat. No.	109843	Neo-Clear® (xylene substitute) for microscopy	5 l
Cat. No.	115943	Methylene blue (C.I. 52015) for microscopy Certistain®	25 g, 100 g
Cat. No.	116450	AFB-Color staining kit for the microscopic investigation of acid-fast bacteria (AFB) (cold staining)	1 set

Hazard classification

Cat. No. 115937

Please observe the hazard classification printed on the label and the information given in the safety data sheet.
The safety data sheet is available on the website and on request.
CAUTION! Contains CMR substances. Please observe the corresponding safety instructions given in the safety data sheet.

Main components of the product

Cat. No. 115937

C.I. 42510 > 80 %
$C_{20}H_{20}ClN_3$
M = 337.85 g/mol

Other IVD products

Cat. No.	101287	Löffler's methylene blue solution for microscopy	100 ml, 500 ml, 2.5 l
Cat. No.	101597	AFB-Fluor phenol-free Staining kit for the examination of acid-fast bacteria with fluorescence microscopy (Auramin-Rhodamine staining)	1 set
Cat. No.	101603	Gram-Color modified (phenol-free) staining kit for Gram staining method on bacteriological smears	1 set
Cat. No.	109093	AFB-Fluor Staining kit for fluorescence-microscopic detection of acid fast bacteria	6x 500 ml
Cat. No.	109204	Giemsa's azur eosin methylene blue solution for microscopy	100 ml, 500 ml, 1 l, 2.5 l
Cat. No.	111609	Histosec® pastilles solidification point 56-58°C embedding agent for histology	1 kg, 10 kg (4x 2.5 kg), 25 kg
Cat. No.	111885	Gram-Color stain set for the Gram staining method	1 set

General remark

If during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and/or its authorised representative and to your national authority.

Literature

1. Romeis - Mikroskopische Technik, Editors: Maria Mulisch, Ulrich Welsch, 2015, Springer Spektrum, 19. Auflage
2. Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble, 6th Edition
3. Histological and Histochemical Methods, Theory and practice, J. A. Kiernan, 2015, Scion Publishing Ltd, 5th Edition
4. Conn's Biological Stains: A Handbook of Dyes, Stains and Fluorochromes for Use in Biology and Medicine, 10th Edition, (ed. Horobin, R.W. and Kiernan, J.A). Bios, 2002


Consult instructions for use


Manufacturer

Catalog number

Batch code


Caution, consult accompanying documents


Use by YYYY-MM-DD


Temperature limitation

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