1.08512.2507 REF

Microscopy

AFB-Color carbol fuchsin solution

for the microscopic investigation of acid-fast bacteria (AFB) (cold staining)

For professional use only

IVD *In Vitro* Diagnostic Medical Device CE

Intended purpose

This "AFB-Color carbol fuchsin solution - for the microscopic investigation of acid-fast bacteria (AFB) (cold staining)" 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 ready-to-use staining solution that when used together with other *in vitro* diagnostic products from our portfolio makes target structures evaluable for diagnostic purposes (e.g. acid-fast bacteria (AFB)) by fixing, embedding, staining, 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. The most efficient staining method is the hot staining according to Ziehl-Neelsen. In this method, carbolfuchsin solution is applied to the specimen and heated. This heating process accelerates the rate at which the fuchsin dye is absorbed and thus also that of the formation of the mycolate-fuchsin complex in the cell wall. The AFB-Color Kit offers a modified AFB-Color Carbol fuchsin solution which abolishes heating (Kinyoun method, cold staining).
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 this instruction for use malachite green or methylene blue is used for counterstaining.
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, exudates, liquid and solid cultures

Sections of formalin-fixed tissue of human origin, embedded in paraffin (3 - 5 µm thick paraffin sections)

Reagents

Cat. No. 1.08512
AFB-Color carbol fuchsin solution 2.5 l
for the microscopic investigation of acid-fast bacteria (AFB) (cold staining)

Also required:

Cat. No. 1.00327	Hydrochloric acid in ethanol for microscopy	1 l, 5 l
Cat. No. 1.01287	Löffler's methylene blue solution for microscopy	100 ml, 500 ml, 2.5 l
Cat. No. 1.08000	Sputofluol™ for microscopy	1 l
Cat. No. 1.10630	AFB-Color malachite green (oxalate) solution for the microscopic investigation of acid-fast bacteria (AFB) (cold staining)	500 ml

Alternatively:

Instead of the combination of single reagents, the staining kit 1.16450.0001 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)

Alternatively for histology:

Cat. No. 1.32450 AFB staining kit for histology 1 set
for the detection of acid-fast bacteria in histological tissue

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

AFB-Color carbol fuchsin solution can be used to stain 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 of smear samples

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

The AFB-Color carbol fuchsin solution - for the microscopic investigation of acid-fast bacteria (AFB) (cold staining) used for staining is ready-to-use, dilution of the solution is not necessary and merely produces a deterioration of the staining result and the stability.

Procedure

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

Staining of smear samples

Staining on the staining rack

Slide with fixed smear		
AFB-Color carbol fuchsin solution	cover completely and stain	20 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	
AFB-Color malachite green (oxalate) solution	cover completely and leave to react	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

Staining in the staining cell

The slides must be immersed and moved about in the solutions, simple immersion alone yields inadequate staining results.

The slides should be allowed to drip off well after the individual staining steps as a measure to avoid any unnecessary cross-contamination of solutions.

Slide with fixed smear	
AFB-Color carbol fuchsin solution	20 min
Running tap water	45 sec
Hydrochloric acid in ethanol	15 - 30 sec*
Running tap water	15 sec
AFB-Color malachite green (oxalate) solution	5 min
Wash with tap water	10 sec
Mount with Aquatex® and cover glass.	

* depending on thickness of specimen

Staining in the automatic stainer

The slides should be allowed to drip off well after the individual staining steps as a measure to avoid any unnecessary cross-contamination of solutions.

Reagent	**Time**	**Station**
Slide with fixed smear		
AFB-Color carbol fuchsin solution*	20 min	1
Wash with tap water	45 sec	5
Hydrochloric acid in ethanol	15 sec	2
Wash with tap water	15 sec	5
AFB-Color malachite green (oxalate) solution	60 sec	3
Wash with tap water	10 sec	5
Air-dry (e.g. 3 min in station 6 or over night or at 50 °C in the drying cabinet)		

* Change reagent at station 1 after 10 - 15 cycles or as required.

Covering with non-aqueous mounting media (e.g. Neo-Mount™, Entellan™ new, or DPX new) and a cover glass is recommended for the storage of bacteriological specimens for several months. For this purpose, the stained specimens must be dried very well.

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

Result

Acid-fast bacteria	red
Background	light green

Staining of histological samples

Staining in the staining cell

Deparaffinize histological slides in the conventional manner and rehydrate in a descending alcohol series.

The slides must be immersed and moved briefly about in the solutions, simple immersion alone yields inadequate staining results.

The slides should be allowed to drip off well after the individual staining steps as a measure to avoid any unnecessary cross-contamination of solutions.

Slide with histological tissue	
AFB-Color carbol fuchsin solution	30 min
Running tap water	45 sec
Hydrochloric acid in ethanol	15 sec
Running tap water	15 sec
Löfflers methylene blue solution*	5 min
Wash with tap water	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
Xylene or Neo-Clear™	5 min
Xylene or Neo-Clear™	5 min
Mount the Neo-Clear™-wet slides with Neo-Mount™ or the xylene-wet slides with e.g. Entellan™ new and cover glass.	

* For the counterstaining of histological specimens a dilution of Löffler's methylene blue solution is recommended (dilution: 1:10 (1+9) with distilled water). In contrast to malachite green, the methylene blue dye is not washed out of the tissue during the dehydration step.
  This working solution can be used for up to 2 weeks.

Result

Acid-fast bacteria	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

Fixing 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.

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.

Too strong background staining

If the background of the histological tissue is stained too strong (dark blue), a dilution of Löffler's methylene blue solution is recommended (dilution: 1:10 (1+9) with distilled water).
Especially compact tissue displays a tendency to over-stain, compared to "more loose" tissues such as e.g. lung tissue, which will need a longer incubation time (5 min like indicated).

Precipitation in the staining solution

In the event that precipitation occurs in the AFB-Color carbol fuchsin solution, the solution must be filtered before use.

Technical notes

The microscope used should meet the requirements of a medical diagnostic laboratory.
When using automatic staining systems, please follow the instructions for use supplied by the supplier of the system and software.
Remove surplus immersion oil before filing.

Analytical performance characteristics

"AFB-Color carbol fuchsin solution" stains and thereby visualizes biological structures, as described in the "Result" chapters of this IFU. The use of the product is only to be carried out by authorized and qualified persons, this includes, among other things, sample and reagent preparation, sample handling, histoprocessing, decisions regarding suitable controls and more.

The analytical performance of the product is confirmed by testing each production batch. The successful participation in international interlaboratory tests on a regular basis provide an additional and unaffiliated confirmation of analytical specificity and repeatability.

For the following stains, the analytical performance was confirmed in terms of specificity, sensitivity and repeatability of the product with a rate of 100 %:

	Inter-assay Specificity	Inter-assay Sensitivity	Intra-assay Specificity	Intra-assay Sensitivity
Kinyoun staining				
Acid-fast bacteria	11/11	11/11	5/5	5/5
Background	11/11	11/11	5/5	5/5

Analytical performance results

Intra- (performed on the same batch) and inter-assay (performed on different batches) data list the number of correctly stained structures in relation to the number of performed assays.

Clinical performance characteristics

The AFB-Color carbol fuchsin solution has been successfully used in the clinical setting for decades in a high number of applications.

The clinical performance of the AFB-Color carbol fuchsin solution in particular was determined by establishing its sensitivity and specificity and its PPV and NPV in an in-house study:

	Ziehl-Neelsen (Kinyoun) Staining
Sensitivity	13/14
Specificity	15/15

Sensitivity: 13 samples out of 14: 92.9 %

Specificity: 15 samples out of 15: 100 %

Positive predictive value (PPV): 100 %

Negative predictive value (NPV): 98.3 %

The results of this Performance Evaluation confirms that the product is suitable for the intended use and performs reliably.

The diagnostic interpretation of the staining results, however, is to be carried out by qualified and authorized professionals, taking into account patient anamnesis, morphology, the use of adequate controls, and additional diagnostic tests, if appropriate. This method can be supplementarily used in human diagnostics.

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 should be conducted with each application in order to avoid an incorrect result.

Storage

Store the AFB-Color carbol fuchsin solution - for the microscopic investigation of acid-fast bacteria (AFB) (cold staining) at +15 °C to +25 °C.

Shelf-life

The AFB-Color carbol fuchsin solution - for the microscopic investigation of acid-fast bacteria (AFB) (cold staining) 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 +15 °C to +25 °C.

The bottles must be kept tightly closed at all times.

Capacity

approx. 250 stainings / 500 ml

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.	1.00327	Hydrochloric acid in ethanol for microscopy	1 l, 5 l
Cat. No.	1.00579	DPX new non-aqueous mounting medium for microscopy	500 ml
Cat. No.	1.00869	Entellan™ new for cover slipper for microscopy	500 ml
Cat. No.	1.01287	Löffler's methylene blue solution for microscopy	100 ml, 500 ml, 2.5 l
Cat. No.	1.01597	AFB-Fluor phenol-free Staining kit for the examination of acid-fast bacteria with fluorescence microscopy (Auramin-Rhodamine staining)	1 set
Cat. No.	1.04699	Immersion oil for microscopy	100-ml dropping bottle, 100 ml, 500 ml
Cat. No.	1.07961	Entellan™ new rapid mounting medium for microscopy	100 ml, 500 ml, 1 l
Cat. No.	1.08000	Sputofluol™ for microscopy	1 l
Cat. No.	1.08298	Xylene (isomeric mixture) for histology	4 l
Cat. No.	1.08562	Aquatex® (aqueous mounting agent) for microscopy	50-ml dropping bottle
Cat. No.	1.09016	Neo-Mount™ anhydrous mounting medium for microscopy	100-ml dropping bottle, 500 ml
Cat. No.	1.09843	Neo-Clear™ (xylene substitute) for microscopy	5 l
Cat. No.	1.10630	AFB-Color malachite green (oxalate) solution for the microscopic investigation of acid-fast bacteria (AFB) (cold staining)	500 ml
Cat. No.	1.16450	AFB-Color staining kit for the microscopic investigation of acid-fast bacteria (AFB) (cold staining)	1 set
Cat. No.	1.32450	AFB staining kit for histology for the detection of acid-fast bacteria in histological tissue	1 set

Hazard classification

Cat. No. 1.08512

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. 1.08512

C.I. 42520 or 42510	6 g/l*
C_6H_5OH	40 g/l

1 l = 0.99 kg

*Both dyes can be used for the preparation of a solution, resulting in the same staining sensitivity and specificity.

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: Mulisch, Maria, Welsch, Ulrich, 2015, Springer-Verlag Berlin Heidelberg, 19. Auflage
2. Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble, 6th Edition
3. 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
4. Kurzlehrbuch Medizinische Mikrobiologie und Infektiologie, Editor: Uwe Groß, Thieme, 2009 2. Auflage

H226: Flammable liquid and vapor.
H314: Causes severe skin burns and eye damage.
H341: Suspected of causing genetic defects.
H351: Suspected of causing cancer.
H373: May cause damage to organs (Nervous system, Kidney, Liver, Skin) through prolonged or repeated exposure.
H410: Very toxic to aquatic life with long lasting effects.

P201: Obtain special instructions before use.
P202: Do not handle until all safety precautions have been read and understood.
P210: Keep away from heat/ sparks/ open flames/ hot surfaces. No smoking.
P233: Keep container tightly closed.
P240: Ground/bond container and receiving equipment.
P241: Use explosion-proof electrical/ventilating/ lighting equipment.
P242: Use non-sparking tools.
P243: Take action to prevent static discharges.
P260: Do not breathe dust/ fume/ gas/ mist/ vapors/ spray.
P264: Wash skin thoroughly after handling.
P273: Avoid release to the environment.
P280: Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301 + P330 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353: Take off immediately all contaminated clothing. Rinse skin with water/ shower.
P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
P305 + P351 + P338+310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.
P308 + P313: IF exposed or concerned: Get medical advice/ attention.
P363: Wash contaminated clothing before reuse.
P370 + P378: In case of fire: Use dry sand, dry chemical or alcoholresistant foam to extinguish.
P391: Collect spillage.
P403 + P235: Store in a well-ventilated place. Keep cool.
P405: Store locked up.
P501: Dispose of contents/container to an approved waste disposal plant.

Revision History

Version	Modification Comment
2024-Jul-22	Initial version with the introduction of Revision History


Consult instructions for use


Manufacturer

Catalog number

Batch code


Caution, consult accompanying documents


Use by YYYY-MM-DD


Temperature limitation

Status: 2024-Jul-22

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