

1.10630.0507

Microscopy

AFB-Color malachite green (oxalate) solution

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

For professional use only



In Vitro Diagnostic Medical Device



Intended purpose

This "AFB-Color malachite green (oxalate) 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 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 (acid-fast bacteria (AFB)) by fixing, staining, counterstaining, mounting) in bacteriological specimen materials, for example smears of enriched bacterial cultures.

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

Acid-fast bacteria are difficult to stain because of the high proportion of lipid and wax in their cell walls. In order to accelerate the absorption of the fuchsin dye and thus the formation of the mycolate-fuchsin complex in the cell wall, the carbol fuchsin solution applied to the preparation is normally heated until it forms steam. In this case, a modification of the AFB-Color carbol fuchsin solution renders heating unnecessary (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 is used for counterstaining. Pretreatment of the specimens with Sputofluo™ 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 Sputofluo™ like sputum, smears from fine needle aspiration biopsies (FNAB), rinses, imprints, effusions, pus, exudates, liquid and solid cultures

Reagents

Cat. No. 1.10630.0507
AFB-Color malachite green (oxalate) solution 1 l, 5 l
for the microscopic investigation of acid-fast bacteria (AFB)
(cold staining)

Also required:

Cat. No. 1.00327 Hydrochloric acid in ethanol 1 l, 5 l
for microscopy
Cat. No. 1.08000 Sputofluo™ 1 l
for microscopy
Cat. No. 1.08512 AFB-Color carbol fuchsin solution 500 ml, 2.5 l
for the microscopic investigation of
acid-fast bacteria (AFB) (cold staining)

Alternatively:

Instead of the combination of single reagents, the staining kit 1.16450.0001 can be used:

Cat. No. 1.16450.0001 1 set
AFB-Color staining kit
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 Sputofluo™ 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 Sputofluo™ solution 15 %

For preparation of approx. 100 ml solution mix:

Sputofluo™	15 ml
Distilled water	85 ml

Preparing sample material in centrifuge tubes:	
Sample	1 part (min. 2 ml)
Sputofluo™ 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.

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 malachite green (oxalate) 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 for staining of smear specimens

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

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

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.

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.

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 malachite green (oxalate) solution" stains and thereby visualizes biological structures, as described in the "Result" chapter 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, decisions regarding suitable controls and more.

The analytical performance of the product is confirmed by testing each production batch.

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
AFB staining				
Acid-fast bacteria	11/11	11/11	8/8	8/8
Background	11/11	11/11	8/8	8/8

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.

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

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 malachite green (oxalate) solution - for the microscopic investigation of acid-fast bacteria (AFB) (cold staining) at +15 °C to +25 °C.

Shelf-life

The AFB-Color malachite green (oxalate) 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. 1.03699	Immersion oil Type N acc. to ISO 8036 for microscopy	100-ml dropping bottle
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	Sputofluo™ for microscopy	1 l
Cat. No. 1.08298	Xylene (isomeric mixture) for histology	4 l
Cat. No. 1.08512	AFB-Color carbol fuchsin solution for the microscopic investigation of acid-fast bacteria (AFB) (cold staining)	500 ml, 2.5 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. 1.16450	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



H361: Suspected of damaging fertility or the unborn child.

P201: Obtain special instructions before use.

P202: Do not handle until all safety precautions have been read and understood.

P280: Wear protective gloves/ protective clothing/ eye protection/ face protection.

P308 + P313: IF exposed or concerned: Get medical advice/ attention.

P405: Store locked up.

P501: Dispose of contents/ container to an approved waste disposal plant.

Hazard classification

Cat. No. 1.10630.0507

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. 1.10630.0507

C.I. 42000 2 g/l

$(C_{23}H_{25}N_2)_2C_2H_4(C_2H_2O_4)_2$

1 l = 1.00 kg

Other IVD products

Cat. No. 1.00496	Formaldehyde solution 4%, buffered, pH 6.9 (approx. 10% Formalin solution) for histology	350 ml and 700 ml (in bottle with wide neck), 5 l, 10 l, 10 l Titripac®
Cat. No. 1.05174	Hematoxylin solution modified acc. to Gill III for microscopy	500 ml, 1 l, 2.5 l
Cat. No. 1.09204	Giemsa's azur eosin methylene blue solution for microscopy	100 ml, 500 ml, 1 l, 2.5 l
Cat. No. 1.09215	Ziehl-Neelsen carbofuchsin solution for microscopy	100 ml, 500 ml, 2.5 l
Cat. No. 1.11609	Histosec™ pastilles solidification point 56-58°C embedding agent for histology	1 kg, 10 kg (4x 2.5 kg), 25 kg
Cat. No. 1.11885	Gram-Color stain set for the Gram staining method	1 set
Cat. No. 1.15355	CYTOCOLOR™ Cytological standard stain acc. to Szczepanik for microscopy	6x 500 ml

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

- Romeis - Mikroskopische Technik, Editors: Mulisch, Maria, Welsch, Ulrich, 2015, Springer-Verlag Berlin Heidelberg, 19. Auflage
- Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble, 6th Edition
- 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

Status: 2023-Feb-09

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