Sigma-Aldrich®

1.09093.0001

Microscopy

AFB-Fluor

Staining kit for fluorescence-microscopic detection of acid-fast bacteria

For professional use only

IVD In Vitro Diagnostic Medical Device

CE

Intended purpose

This "AFB-Fluor - Staining kit for fluorescence-microscopic detection of acid-fast bacteria" is used for human-medical cell diagnosis and serves the purpose of the bacteriological, cytological, and histological investigation of sample material of human origin. It is a ready-to-use staining kit that when used together with other in vitro diagnostic products from our portfolio makes bacterial target structures evaluable for diagnostic purposes (acid-fast bacteria (AFB)) by fixing, embedding, staining, counterstaining, mounting in bacteriological, cytological, and histological specimen materials, for example smears of enriched bacterial cultures or histological sections of e.g. the lung.

The decisive feature of the AFB-Fluor staining kit is the presence of fluorescence dyes in the staining solution. This enables the swift and secure identification of the acid-fast bacteria under the fluorescence microscope. The sensitivity and specificity of the staining results are identical to those for the staining method of the AFB-Fluor phenol-free (Cat. No. 101597).

This staining set is supplied ready-for-use and contains all the reagents required for the staining of acid-fast bacteria in smear specimens and in histological tissue specimens.

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 classic Ziehl-Neelsen method is determined by the high proportion of wax and lipids in the cell wall, since acid-fast bacteria take up staining 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 staining solution applied to the specimen is normally heated to evaporation point.
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.
When using the AFB-Fluor staining kit, the use of the fluorescent dyes auramine and rhodamine in the AFB-Fluor staining solution render the heating step unnecessary. These dyes deposit on the cell wall of the acid-fast bacteria and, depending on the microscope filter, appear yellow or red against a black background.
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. 1.09093.0001
AFB-Fluor
Staining kit for fluorescence-microscopic detection of acid-fast bacteria

Package components:

The staining kit contains

Reagent 1:	Auramine-rhodamine staining solution	500 ml
Reagent 2:	Decolorizing solution	3 x 500 ml
Reagent 3:	Counterstaining solution ($KMnO_4$)	2 x 500 ml

Also required:

Cat. No. 108000	Sputofluol® for microscopy	1 l

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	

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

AFB-Fluor can be used to stain histological sections.

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

Fixation of smear samples

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.

Reagent preparation

The reagents 1, 2, and 3 of the AFB-Fluor - Staining kit for fluorescence-microscopic detection of acid-fast bacteria used for staining are ready-to-use, dilution of the solutions is not necessary and merely produces a deterioration of the staining result and their stability.

Due to its high viscosity, the auramine-rhodamine staining solution (Reagent 1) must be vigorously shaken before use.

Procedure

Staining of smear samples

Staining in the staining cell

The slides must be immersed and moved briefly 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.

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

Slide with fixed smear	
Reagent 1 (Auramine-rhodamine staining solution)	15 min
Running tap water	10 min
Reagent 2 (Decolorizing solution)	1 min
Running tap water	5 min
Reagent 3 (Counterstaining solution)	5 min
Running tap water	5 min
Air-dry	

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.

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

	Station	Time	Dip mode
Slide with fixed smear			
Reagent 1 (Auramine-rhodamine staining solution)	4	15 min	-
Running tap water	5	10 min	+
Reagent 2 (Decolorizing solution)	3	1 min	+
Running tap water	5	5 min	+
Reagent 3 (Counterstaining solution)	2	5 min	-
Running tap water	5	5 min	+
Dry	6	5 min	

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 smear samples for several months. For this purpose, the stained specimens must be dried very well. 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.

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.

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

Slide with histological tissue	
Distilled water	rinse
Reagent 1 (Auramine-rhodamine staining solution)	15 min
Running tap water	10 min
Reagent 2 (Decolorizing solution)	1 min
Running tap water	5 min
Reagent 3 (Counterstaining solution)	5 min
Running tap water	5 min
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.	

After dehydration (ascending alcohol series) and clarification with xylene or Neo-Clear®, histological 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.

Result

Acid-fast bacteria (in the fluorescence microscope)	red-orange (rhodamine) or yellow-green (auramine) depending on the combination of filters used
Background	dark to black

Staining for optical and fluorescence microscopy

The fluorescent stain using the AFB-Fluor staining kit is also ideally suited for the additional staining of the acid-fast bacteria for diagnosis by optical microscopy, e.g. using the AFB-Color staining kit (Cat. No. 116450) or AFB-Color modified (Cat. No. 100497).

The unmounted specimens stained with AFB-Fluor staining kit can be used directly for diagnostic purposes. Subsequently, after carefully removing any immersion oil that may have been used, the specimens can be further stained with the AFB-Color staining kit or AFB-Color modified.
In the specimen treated in this manner, the acid-fast bacteria appear magenta-red against a light green (AFB-Color) or light blue (AFB-Color modified) background under the optical microscope.

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.

If the result is not conclusive, it is recommended to additionally stain the specimens for further optical-microscopy diagnosis, e. g. with the AFB-Color staining kit (Cat. No. 116450) or AFB-Color modified (Cat. No. 100497).

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

In the event that the background is too strongly stained in the optical microscopic image (dark blue), Reagent 1 (auramine-rhodamine staining solution) should be better mixed and subsequently better decolorized. Since the staining solution is highly viscous, it is recommended to swirl the slide during the decolorizing step with Reagent 2 (decolorizing solution).

Technical note

The microscope used should meet the requirements of a medical diagnostic laboratory.

Recommended filter combination for fluorescence microscopy:

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

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.

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.
If the result is not conclusive, it is recommended to additionally stain the specimens for further optical-microscopy diagnosis, e. g. with the AFB-Color staining kit (Cat. No. 116450) or AFB-Color modified (Cat. No. 100497).

Storage

Store the AFB-Fluor - Staining kit for fluorescence-microscopic detection of acid-fast bacteria at +15 °C to +25 °C.

Shelf-life

The AFB-Fluor - Staining kit for fluorescence-microscopic detection of acid-fast bacteria 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

The package is sufficient for up to 300 applications.

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.	100327	Hydrochloric acid in ethanol for microscopy	1 l, 5 l
Cat. No.	100496	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.	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.	100869	Entellan® new for cover slipper for microscopy	500 ml
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.	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.	103999	Formaldehyde solution min. 37% free from acid stabilized with about 10% methanol and calcium carbonate for histology	1 l, 2.5 l, 25 l
Cat. No.	104699	Immersion oil for microscopy	100-ml dropping bottle, 100 ml, 500 ml
Cat. No.	107164	Paraffin pastilles solidification point about 56-58°C for histology	10 kg (4x 2.5 kg)
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.	108562	Aquatex® (aqueous mounting agent) for microscopy	50-ml dropping bottle
Cat. No.	108298	Xylene (isomeric mixture) for histology	4 l
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.	111609	Histosec® pastilles soldification point 56-58°C embedding agent for histology	1 kg, 10 kg (4x 2.5 kg), 25 kg
Cat. No.	115161	Histosec® pastilles (without DMSO) soldification point 56-58°C embedding agent for histology	10 kg (4x 2.5 kg), 25 kg
Cat. No.	116450	AFB-Color staining kit for the microscopic investigation of acid-fast bacteria (AFB) (cold staining)	1 set

Hazard classification
Cat. No. 1.09093.0001

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 product components
Cat. No. 1.09093.0001

Reagent 1

C.I. 41000	12.0 g/l
C.I. 45170	6.0 g/l

Reagent 2

C_3H_8O	543.3 g/l
HCl min. 37 %	15.7 g/l

Reagent 3

$KMnO_4$	5.0 g/l

Other IVD products

Cat. No.	101603	Gram-Color modified (phenol-free) staining kit for Gram staining method on bacteriological smears	1 set
Cat. No.	102473	ISOSLIDE® Methenamine Control Slides with reference tissue for the detection of argent-affine structures in histological tissue	25 tests
Cat. No.	102561	ISOSLIDE® Congo Red Control Slides with reference tissue for the detection of amyloid structures in histological tissue	25 tests
Cat. No.	109215	Ziehl-Neelsen carbolfuchsin solution for microscopy	100 ml, 500 ml, 2.5 l
Cat. No.	111885	Gram-Color stain set for the Gram staining method	1 set
Cat. No.	115525	RINGER tablets for the preparation of RINGER'S solution	100 tabs
Cat. No.	132450	AFB staining kit for histology for the detection of acid-fast bacteria in histological tissue	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: 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. Routine Cytological Staining Techniques: Theoretical Background and Practice, Mathilde E. Boon, Johanna S. Drijver, 1986, Elsevier Science Publishing company


Consult instructions for use


Manufacturer

Catalog number

Batch code


Caution, consult accompanying documents


Use by YYYY-MM-DD


Temperature limitation

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