1.15940.0025
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Microscopy

Crystal violet (C.I. 42555)

for microscopy Certistain®

For professional use only

IVD In Vitro Diagnostic Medical Device

CE

Intended purpose

This staining dye "Crystal violet (C.I. 42555) - for microscopy Certistain®" is used for human-medical cell diagnosis and serves the purpose of the bacteriological 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 in bacteriological specimen materials evaluable for diagnostic purposes (e.g. Gram-positive or Gram-negative bacteria, by fixing, embedding where necessary, staining with the above crystal violet solution, counterstaining, mounting).

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

In bacteriology, the Gram staining allows a fast differentiation of bacteria in Gram-positive and Gram-negative.

The mureine structure of the bacteria wall is the basis of the color affinity. In the first step, bacteria will be stained with crystal violet, an aniline dye. After the treatment with iodine solution (Lugol's solution), a dye-iodine complex will form. During the decolorizing step, this complex stays in the multi-layer mureine structure of the cell wall of Gram-positive bacteria - they will appear blue-violet.
Gram-negative bacteria, by contrast, have a cell wall consisting of a single-layered murein structure, and correspondingly re-release the staining dye with the decoloring solution. Gram-negative bacteria will be counterstained with safranine solution and will then appear pink to red.

Sample material

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

Reagents

Cat. No. 115940
Crystal violet (C.I. 42555)
for microscopy Certistain® — 25 g, 100 g, 1 kg

Color Index No.: 42555
Color Index Name: Basic violett 3

Also required:

Cat. No. 100983 Ethanol absolute
for analysis EMSURE® ACS,ISO,Reag. Ph Eur — 1 l, 2.5 l, 5 l

Cat. No. 101192 di-Ammonium oxalate monohydrate
for analysis EMSURE® ACS,ISO,Reag. Ph Eur — 250 g, 1 kg

Cat. No. 100567 Lugol's solution stabilized with PVP
for the Gram staining method — 1 l, 2.5 l

or

Cat. No. 109261 Lugol's solution (diluted iodine-potassium iodide solution)
for the Gram staining method — 1 l, 2.5 l

Cat. No. 109217 Gram's safranine solution
for the Gram staining method — 500 ml, 2.5 l

Cat. No. 110218 Gram's decolorizing solution
for the Gram staining method — 500 ml, 2.5 l

Alternatively:

Cat. No. 109218 Gram's crystal violet solution
for the Gram staining method — 500 ml, 2.5 l

Instead of the combination of single reagents, the staining kit 1.11885.0001 can be used:

Cat. No. 1.11885.0001
Gram-color
stain set for the Gram staining method — 1 set

Sample preparation

The sampling must be performed by qualified personnel.

Apply the specimen material to a clean and grease-free slide using an annealed loop. Then smear the material either directly onto the slide or first mix with 1 - 2 drops of physiological saline solution (Ringer's solution). Air-dry and then heat-fix by slowly drawing the slide (smear side facing up) through the upper part of the Bunsen-burner flame for three times. Subsequently, allow to cool and stain.

The air-dried smears must be heat-fixed very carefully. This prevents the risk of infections and reduces the dissolution of specimen material and thus, the contamination of solutions and other slides.

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.

Reagent preparation

Staining can be carried out using either a ready-to-use (Cat. No. 109218) or a separately prepared crystal violet solution (prepared from Cat. No. 115940).

Ammonium oxalate solution 1 %, aqueous

For preparation of approx. 100 ml solution mix and dissolve:

di-Ammonium oxalate monohydrate	1.0 g
Distilled water	99 ml
mix	

Crystal violet solution

For preparation of approx. 100 ml solution mix:

Crystal violet (C.I. 42555) Certistain®	2.0 g
Ethanol 96 %	20 ml
dissolve	
Ammonium oxalate solution 1 %, aqueous	80 ml
mix and filter	

The freshly prepared staining solution should be filtered before use.

It is recommended to dilute the crystal violet solution 1+4 with distilled water, if the immersion method is used.

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		
Crystal violet solution	cover completely and leave to react	1 min
Lugol's solution	rinse briefly	
Lugol's solution	cover completely and leave to react	1 min
Distilled water	wash carefully	5 sec
Gram's decolorizing solution	carefully swirl the slides until no further clouds of dye are produced and the smear takes on a grey-blue color	10 - 15 sec
Distilled water	wash carefully	5 sec
Gram's safranine solution	cover completely and leave to react	1 min
Distilled water	wash carefully	5 sec
Air-dry (e.g. over night or at 50 °C in the drying cabinet)		

Covering with non-aqueous mounting media (e.g. Neo-Mount® or Entellan®) 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. 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

Gram-positive microorganisms	blue-violet
Gram-negative microorganisms	pink to red

Trouble-shooting

Fixing

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 the gram-positive bacteria

The critical stage of the Gram-staining procedure is the decolorizing step, which can be influenced by the thickness of the smear. In addition, a freshly prepared solution is highly reactive, which is why the result should be evaluated with care. During the decolorizing step, the user should stick to the exact incubation times described in the protocol, since otherwise false-negative results may result.

Technical notes

The microscope used should meet the requirements of a medical diagnostic laboratory.
The freshly prepared staining solution 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 should be conducted with each application in order to avoid an incorrect result.
The control may be performed with Gram-positive bacteria (e.g. staphylococci) and Gram-negative bacteria (e.g. Escherichia coli).
Bacteria taken from a culture medium after 18 - 24 hours of incubation should be used.

Storage

Store Crystal violet (C.I. 42555) - for microscopy Certistain® at +5 °C to +30 °C.

Shelf-life

Crystal violet (C.I. 42555) - 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.	100567	Lugol's solution stabilized with PVP for the Gram staining method	1 l, 2.5 l
Cat. No.	100983	Ethanol absolute for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l, 5 l
Cat. No.	101192	di-Ammonium oxalate monohydrate for analysis EMSURE® ACS,ISO,Reag. Ph Eur	250 g, 1 kg
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.	109016	Neo-Mount® anhydrous mounting medium for microscopy	100-ml dropping bottle, 500 ml
Cat. No.	109217	Gram's safranine solution for the Gram staining method	500 ml, 2.5 l
Cat. No.	109218	Gram's crystal violet solution for the Gram staining method	500 ml, 2.5 l
Cat. No.	109261	Lugol's solution (diluted iodine-potassium iodide solution) for the Gram staining method	1 l, 2.5 l
Cat. No.	110218	Gram's decolorizing solution for the Gram staining method	500 ml, 2.5 l
Cat. No.	111885	Gram-color stain set for the Gram staining method	1 set

Hazard classification

Cat. No. 115940

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. 115940

C.I. 42555 > 88 %

$C_{25}H_{30}ClN_3$

M = 407.99 g/mol

Other IVD products

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.	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.	109843	Neo-Clear® (xylene substitute) for microscopy	5 l
Cat. No.	115525	RINGER tablets for the preparation of RINGER'S solution	100 tabs
Cat. No.	116450	AFB-Color staining kit for the microscopic investigation of acid-fast bacteria (AFB) (cold staining)	1 set
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. 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
2. Romeis - Mikroskopische Technik, Editors: Maria Mulisch, Ulrich Welsch, 2015, Springer Spektrum, 19. Auflage
3. Theory and Practice of Histological Techniques, John D Bancroft, Marilyn Gamble, 2008, Churchill Livingstone ELSEVIER, sixth Edition
4. Histological and Histochemical Methods, Theory and practice, J. A. Kiernan, 2015, Scion Publishing Ltd, 5th Edition
5. Laboratory Manual of Histochemistry, Linda L. Vacca, 1985, Raven Press
6. Staining Procedures, George Clark, 1981, Williams&Wilkins, fourth Edition
7. Kurzlehrbuch Medizinische Mikrobiologie und Infektiologie Uwe Groß, Thieme, 2009 2. Auflage


Consult instructions for use


Manufacturer

Catalog number

Batch code


Caution, consult accompanying documents


Use by YYYY-MM-DD


Temperature limitation

Status: 2021-Jul-09

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