1.11885.0001

Microscopy

Gram-Color

stain set for the Gram staining method

For professional use only

IVD In Vitro Diagnostic Medical Device

CE

Intended purpose

This "Gram-Color - stain set for the Gram staining method" 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 kit that when used together with other in vitro diagnostic products from our portfolio makes bacterial target structures evaluable for diagnostic purposes (Gram-positive or Gram-negative bacteria) by fixing, staining, counterstaining, mounting in bacteriological specimen materials, e. g. smears of body fluids.

The Gram-Color solutions are modified and designed in such a way that staining can be carried out in staining cells, on the staining rack, and also in automated staining systems.

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 multilayer 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. 1.11885.0001
Gram-Color
stain set for the Gram staining method

Package components:

The staining kit contains

Reagent 1:	Crystal violet solution	500 ml
Reagent 2:	Lugol's solution, stabilized	500 ml
Reagent 3 = 4:	Decolorizing solution	each 500 ml
Reagent 5:	Safranine solution	500 ml

Single reagents

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

Cat. No.	109218	Gram's crystal violet solution for the Gram staining method	500 ml, 2.5 l
Cat. No.	100567	Lugol's solution stabilized with PVP for the Gram staining method	1 l, 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.	109217	Gram's safranine solution for the Gram staining method	500 ml, 2.5 l

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

The reagents of the Gram-Color - stain set for the Gram staining method used for staining are ready-to-use, dilution of the solutions (except for reagent 1 for staining in the cell - see "Procedure") is not necessary.

Procedure

Staining in the staining cell

It is recommended to dilute the reagent 1 (Gram's crystal violet solution) 1:3 with distilled water, if the immersion method is used.

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.

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

Slide with fixed smear	
Reagent 1 (crystal violet solution)	1:30 min
Running tap water	30 sec
Reagent 2 (Lugol's solution, stabilized)*	3 min
Running tap water	20 sec
Reagent 3 or 4 (decolorizing solution)**	5 - 10 sec
Running tap water	30 sec
Reagent 5 (safranine solution)	1 min
Running tap water	1 min
Air-dry (e. g. over night or at 50 °C in the drying cabinet)	

* filter reagent 2 (Lugol's solution, stabilized) after 3 runs
** discard reagent 3 or 4 (decolorizing solution) after 5 runs

Staining on the staining rack

Slide with fixed smear		
Reagent 1 (crystal violet solution)	cover completely and leave to react	1 min
Reagent 2 (Lugol's solution, stabilized)	rinse briefly	
Reagent 2 (Lugol's solution, stabilized)	cover completely and leave to react	1 min
Distilled water	wash carefully	5 sec
Reagent 3 or 4 (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
Reagent 5 (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.

Staining in the automatic stainer

Staining in automated staining systems can be performed according to the protocol of the staining in the staining cell.

Result

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

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 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 fresh decolorizing 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.
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 should be conducted with each application in order to avoid an incorrect result.
The staining set may be controlled with Gram-positive bacteria and Gram-negative bacteria.
Bacteria taken from a culture medium after 18 - 24 hours of incubation should be used.

Storage

Store the Gram-Color - stain set for the Gram staining method at +15 °C to +25 °C.

At temperatures below 15 °C a colored precipitate may settle out of the staining solutions. If precipitation has occurred, place the bottle for 2 - 3 hours in a water bath set at approx. 60 °C. This will re-dissolve most of the precipitate. Subsequently, filter the staining solutions through a paper filter.

Shelf-life

The Gram-Color - stain set for the Gram staining method 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 250 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.	100567	Lugol's solution stabilized with PVP for the Gram staining method	1 l, 2.5 l
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

Hazard classification

Cat. No. 1.11885.0001

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 components of the products

Cat. No. 1.11885.0001

Reagent 1 or Cat. No. 109218

C.I. 42555	10 g/l
C_6H_5OH	4 g/l

1 l = 0.99 kg

Reagent 2 or Cat. No. 100567

PVP-Iodine	50 g/l
KI	10 g/l

1 l = 1.02 kg

Cat. No. 109261

I_2	3.4 g/l
KI	6.8 g/l

1 l = 1.01 kg

Reagent 3/4 or Cat. No. 110218

C_2H_6O	634 g/l
C_3H_6O	159 g/l

1 l = 0.79 kg

Reagent 5 or Cat. No. 109217

C.I. 50240	1.8 g/l

1 l = 0.98 kg

Other IVD products

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.	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. Romeis - Mikroskopische Technik, Editors: Mulisch, Maria, Welsch, Ulrich, 2015, Springer-Verlag Berlin Heidelberg, 19. Auflage
2. 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
3. Kurzlehrbuch Medizinische Mikrobiologie und Infektiologie, Editor: Uwe Groß, Thieme 2009, 2. Auflage
4. Histological and Histochemical Methods, Theory and practise, J.A. Kiernan, Scion, 5th Editon


Consult instructions for use


Manufacturer



Catalog number



Batch code


Caution, consult accompanying documents


Use by YYYY-MM-DD


Temperature limitation

Status: 2020-Jul-13

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