

1.01603.0001

Microscopy

Gram-Color modified (phenol-free)

staining kit for Gram staining method on bacteriological smears

For professional use only



In Vitro Diagnostic Medical Device



Intended purpose

This "Gram-Color modified (phenol-free) - staining kit for Gram staining method on bacteriological smears" 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 target structures evaluable for diagnostic purposes (Gram-positive or Gram-negative bacteria) by fixing, staining, counterstaining, mounting in bacteriological specimen materials, for example smears of body fluids.

Gram-Color modified is a staining kit which is used for a modified Gram staining.

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

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 decolorizing solution. Gram-negative bacteria will be counterstained with fuchsin solution and will then appear red.

Sample material

Body fluids, exsudate, pus, liquid or solid cultures

Reagents

Cat. No. 1.01603.0001

Gram-Color modified (phenol-free)

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Package components:

The staining kit contains

Reagent 1a:	Crystal violet solution	100 ml
Reagent 1b:	Sodium hydrogen carbonate solution	100 ml
Reagent 1c:	Bottle for Reagent 1c (of 1a und 1b)	
Reagent 2:	Lugol's solution, stabilized	190 ml
Reagent 3:	Decolorizing solution	190 ml
Reagent 4:	Fuchsin solution, (phenol-free)	190 ml

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 2, 3, and 4 of the Gram-Color modified (phenol-free) - staining kit for Gram staining method on bacteriological smears are ready-to-use, dilution of the solutions is not necessary and merely produces a deterioration of the staining result and their stability.

Staining solution (Reagent 1c)

Mix reagent 1a (Crystal violet solution) and reagent 1b (Sodium hydrogen carbonate solution) 1+1 in the bottle provided (1c).

This mixture is sufficient for approximately 65 - 70 specimens and can be stored at room temperature for 10 days and refrigerated 14 days, respectively. If this amount appears to be too large for this period of time, it is advisable to prepare a smaller quantity (approx. 3 ml are needed per microscopic slide).

Procedure

Staining on the staining rack

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

Slide with fixed smear		
Reagent 1c (self-prepared staining solution)	cover completely and leave to react	1 min
Distilled water	rinse carefully	5 sec
Reagent 2 (Lugol's solution, stabilized)	cover completely and leave to react	1 min
Distilled water	rinse carefully	5 sec
Reagent 3 (decolorizing solution)	cover completely	5 - 10 sec
Distilled water	rinse carefully	5 sec
Reagent 4 (fuchsin solution, phenol-free)	cover completely and leave to react	15 - 30 sec
Distilled water	rinse 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®, Entellan®, 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. 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	dark blue
Gram-negative microorganisms	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 modified (phenol-free) - staining kit for Gram staining method on bacteriological smears at +15 °C to +25 °C.

At temperatures below 15 °C a colored precipitate may settle out of the dye 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 modified (phenol-free) - staining kit for Gram staining method on bacteriological smears 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.

Reagent 1c (self-prepared staining solution) can be used up to 10 days when stored at +15 °C to +25 °C and 14 days when stored at +2 °C to +8 °C.

Capacity

The package is sufficient for 65 - 70 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. 100579	DPX new non-aqueous mounting medium for microscopy	500 ml
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 safranin solution	500 ml, 2.5 l
Cat. No. 115525	RINGER tablets for the preparation of RINGER'S solution	100 tabs

Hazard classification

Cat. No. 1.01603.0001

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. 1.01603.0001		
Reagent 1a		
C.I. 42555		10 g/l
1 l = 0.99 kg		
Reagent 1b		
NaHCO ₃		25 g/l
Reagent 2		
PVP-Iodine		50 g/l
KI		10 g/l
1 l = 1.02 kg		
Reagent 3		
C ₂ H ₆ O		634 g/l
C ₃ H ₆ O		159 g/l
1 l = 0.79 kg		
Reagent 4		
C.I. 42510 or 42520*		0.9 g/l
C ₂ H ₆ O		79 g/l

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

Other IVD products

Cat. No. 100327	Hydrochloric acid in ethanol for microscopy	1 l, 5 l
Cat. No. 101287	Löffler's methylene blue solution for microscopy	100 ml, 500 ml, 2.5 l
Cat. No. 108000	Sputofluol® for microscopy	1 l
Cat. No. 109215	Ziehl-Neelsen carbolfuchsin solution for microscopy	100 ml, 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. 111885	Gram-Color stain set for the Gram staining method	1 set
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

- 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
- Kurzlehrbuch Medizinische Mikrobiologie und Infektiologie, Editor: Uwe Groß, Thieme 2009, 2. Auflage
- Histological and Histochemical Methods, Theory and practise, J.A. Kiernan, Scion, 5th Edition



Consult instructions for use



Manufacturer



Catalog number



Batch code



Caution, consult accompanying documents



Use by YYYY-MM-DD



Temperature limitation

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Merck KGaA, 64271 Darmstadt, Germany,
Tel. +49(0)6151 72-2440
www.microscopy-products.com

EMD Millipore Corporation, 400 Summit Drive
Burlington MA 01803, USA, Tel. +1-978-715-4321
Sigma-Aldrich Canada Co. or Millipore (Canada) Ltd.
2149 Winston Park, Dr. Oakville, Ontario, L6H 6J8
Phone: +1 800-565-1400

