1.15925.0025

Microscopy

Orange G (C.I. 16230)

for microscopy Certistain®

IVD In Vitro Diagnostic Medical Device CE

for Papanicolaou's stain in cytology

This staining dye "Orange G (C.I. 16230) - for microscopy Certistain®" is used for human-medical cell diagnosis and serves the purpose of the cytological 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 (by fixing, embedding, staining with the above Orange G solution, counterstaining, mounting) in human gynecological and clinico-cytological specimen materials, for example cervical smears, evaluable for diagnostic purposes.

Principle

Most used staining procedure for cytological specimen is Papanicolaou's technique. It enables an adequate statement about dignity, hormone status, and vaginal flora. In addition, it can also be used for staining specimens for carcinoma diagnosis.

In the first staining step are stained the cell nuclei by a hematoxylin solution. Nuclei are stained blue to dark violet.

The second staining step is cytoplasmic staining by orange staining solution, especially for demonstration of mature and keratinized cells. The target structures are stained orange in different intensities.

In the third staining step is used the so-called polychrome solution, a mixture of eosin, light green SF and Bismarck brown. The polychrome solution is used for demonstration of differentiation of squamous cells.

Sample material

Gynaecological and non-gynaecological specimen as sputum, urine, smears from fine needle aspiration biopsies (FNAB), effusions, rinses

Reagents

Cat. No. 1.15925.0025

Orange G (C.I. 16230) for microscopy Certistain® — 25 g
Color Index No.: 16230
Color Index Name: Acid orange 10

Also required:

Cat. No. 100583 Tungstophosphoric acid hydrate for analysis EMSURE® — 100 g, 250 g

Cat. No. 100974 Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE® — 1 l, 2.5 l

for nucleus staining:

Cat. No. 109253 Papanicolaou's solution 1a Harris hematoxylin solution for cytological cancer and cycle diagnosis — 500 ml, 1 l, 2.5 l

or

Cat. No. 109254 Papanicolaou's solution 1b Hematoxylin solution S for cytological cancer and cycle diagnosis — 500 ml, 2.5 l

for differentiation:

Cat. No. 109271 Papanicolaou's solution 3a polychromatic solution EA 31 for cytological cancer and cycle diagnosis — 500 ml, 2.5 l

or

Cat. No. 109272 Papanicolaou's solution 3b polychromatic solution EA 50 for cytological cancer and cycle diagnosis — 500 ml, 1 l, 2.5 l

Sample preparation

The sampling must be performed by qualified personnel.

Fixation

Wet fixation immediately with spray fixative M-FIX™ for min. 10 min or wet fixation immediately in ethanol 96 % for min. 30 min.
When the smears are fixed with M-FIX™, rinsing steps 1 - 4 in the ascending ethanol sequence prior to staining can be omitted.

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.

Reagent preparation

Orange G working solution

For preparation of approx. 100 ml solution mix:

Orange G (C.I. 16230) Certistain®	0.5 g
Ethanol	100 ml
dissolve	
Tungstophosphoric acid hydrate	0.015 g
add and stir until it is dissolved	

The freshly prepared staining solution should be filtered before use.

Procedure

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 to guarantee an optimal staining result.

Slide with fixed smear	
Ethanol 96 %*	10 sec
Ethanol 80 %*	10 sec
Ethanol 70 %*	10 sec
Ethanol 50 %*	10 sec
Distilled water	20 sec
Papanicolaou's solution 1a Harris hematoxylin solution or Papanicolaou's solution 1b Hematoxylin solution S	3 min
Running tap water	3 min
Ethanol 70 %	30 sec
Ethanol 80 %	30 sec
Ethanol 96 %	30 sec
Orange G working solution	3 min
Ethanol 96 %	30 sec
Ethanol 96 %	30 sec
Papanicolaou's solution 3a polychromatic solution EA 31 or Papanicolaou's solution 3b polychromatic solution EA 50	3 min
Ethanol 96 %	30 sec
Ethanol 96 %	30 sec
Ethanol 100 %	5 min
Mixture consisting of: Ethanol 100 % + Neo-Clear® or xylene (1 + 1)	2 min
Clarify with Neo-Clear® or xylene.	5 min
Clarify with Neo-Clear® or xylene.	5 min
Mount the Neo-Clear®-wet slides with Neo-Mount® or the xylene-wet slides with e.g. Entellan® new and cover glass.	

* These steps can be omitted when smears are fixed with M-FIX™.

After dehydration (ascending alcohol series) and clarification with xylene or Neo-Clear®, cytological samples can be mounted with water-free mounting agents (e.g. Entellan® new, DPX new, or Neo-Mount®) and a cover glass and and can then be stored.

The use of immersion oil is recommended for the analysis of stained slides with a microscopic magnification >40x.

Result

Staining with	3a / EA 31	3b / EA 50
Cytoplasm cyanophilic (basophilic) eosinophilic (acidophilic) keratinized	blue-green to green pink pink-orange	blue-green pink pink-orange
Erythrocytes	red	
Cell nuclei	blue to dark violet	
Microorganisms	grey-blue	
Trichomonas	grey-green	

Technical notes

The microscope used should meet the requirements of a medical diagnostic laboratory.
When using histoprocessor systems or automatic staining systems, please follow the instructions for use supplied by the supplier of the system and software.
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 trained personnel.
Valid nomenclatures must be used.
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 Orange G (C.I. 16230) - for microscopy Certistain® at +5 °C to +30 °C.

Shelf-life

Orange G (C.I. 16230) - 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.	100579	DPX new non-aqueous mounting medium for microscopy	500 ml
Cat. No.	100583	Tungstophosphoric acid hydrate for analysis EMSURE®	100 g, 250 g
Cat. No.	100974	Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 l, 2.5 l
Cat. No.	103981	M-FIX™ spray fixative for cytodiagnosis	100 ml, 1 l
Cat. No.	104699	Immersion oil for microscopy	100-ml dropping bottle, 100ml, 500 ml
Cat. No.	107961	Entellan® new rapid mounting medium for microscopy	100 ml, 500 ml, 1 l
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.	109253	Papanicolaou’s solution 1a Harris hematoxylin solution for cytological cancer and cycle diagnosis	500 ml, 1 l, 2.5 l
Cat. No.	109254	Papanicolaou’s solution 1b Hematoxylin solution S for cytological cancer and cycle diagnosis	500 ml, 2.5 l
Cat. No.	109271	Papanicolaou’s solution 3a polychromatic solution EA 31 for cytological cancer and cycle diagnosis	500 ml, 2.5 l
Cat. No.	109272	Papanicolaou’s solution 3b polychromatic solution EA 50 for cytological cancer and cycle diagnosis	500 ml, 1 l, 2.5 l
Cat. No.	109843	Neo-Clear® (xylene substitute) for microscopy	5 l

Hazard classification

Cat. No. 1.15925.0025

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.

Main components of the product

Cat. No. 1.15925.0025

C.I. 16230
$C_{16}H_{10}N_2Na_2O_7S_2$
M = 452.37 g/mol
Dye content ≥ 80 %

Other IVD products

Cat. No.	109269	Papanicolaou’s solution 3d polychromatic solution EA 65 for cytological cancer and cycle diagnosis	100 ml, 2.5 l
Cat. No.	109270	Papanicolaou’s solution 3c polychromatic solution EA 65 for cytological cancer and cycle diagnosis	100 ml
Cat. No.	109275	Shorr staining solution for hormonal cytodiagnosis	500 ml
Cat. No.	115355	CYTOCOLOR® Cytological standard stain acc. to Szczepanik for microscopy	6x 500 ml

Literature

1. Routine Cytological Staining Techniques: Theoretical Background and Practice, Mathilde E. Boon, Johanna S. Drijver, 1986, Elsevier Science Publishing Company
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


Consult instructions for use


Manufacturer

Catalog number

Batch code


Caution, consult accompanying documents


Use by YYYY-MM-DD


Temperature limitation

Status: 2017-08-15

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

EMD Millipore Corporation, 290 Concord Road, Billerica, MA 01821, USA, Tel. +1-978-715-4321