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Microscopy

Alcian blue 8 GX (C.I. 74240)

for microscopy Certistain®

 In Vitro Diagnostic Medical Device


for detection of acidic mucosubstances

This staining dye "Alcian blue 8 GX (C.I. 74240) - for microscopy Certistain®" is used for human-medical cell diagnosis and serves the purpose of the histological 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 histological specimen materials (by fixing, where necessary embedding, staining with the above alcian blue solution, counterstaining, mounting) evaluable for diagnostic purposes.

Principle

The Alcian blue staining is a good alternative and easily combinable with the Periodic acid-Schiff (PAS) reaction. In the tissue, acid mucosubstances are stained selectively and nuclear fast red is commonly used as counter stain. In combination with the PAS reaction, non-substituted polysaccharides, neutral mucopolysaccharides, muco- and glycoproteins and glycol- and phospholipids can be visualized in addition.

An Acetic acid-Alcian blue solution is used for the staining, whereby carboxylated and sulfatic proteoglycans are selectively stained when a pH of 2.5 is applied. The differentiation between carboxyl and sulfate groups is only possible at pH 1.

Sample material

Sections of formalin-fixed, paraffin-embedded tissue (3 - 5 µm thick paraffin sections) or cell smears are used as starting material.

Reagents

Cat. No. 105234

Alcian blue 8 GX (C.I. 74240) for microscopy Certistain® 10 g

Color Index No.: 74240

Color Index Name: Ingrain blue 1

Also required:

Cat. No. 100063 Acetic acid (glacial) 100% anhydrous 1 l, 2.5 l
for analysis EMSURE® ACS,ISO,Reag. Ph Eur

for Alcian blue staining:

Cat. No. 100121 Nuclear fast red-aluminum sulfate solution 0.1 % 500 ml
for microscopy

for Alcian blue-PAS staining:

Cat. No. 101646 PAS staining kit for detection of aldehyde and mucosubstances 2x 500 ml

Cat. No. 105174 Hematoxylin solution modified acc. to Gill III 500 ml, 1 l, 2.5 l
for microscopy

Optional (s. "Alcian blue-PAS staining - Procedure", footnotes):

Cat. No. 105175 Hematoxylin solution modified acc. to Gill II 500 ml, 2.5 l
for microscopy

Cat. No. 106528 Sodium disulfite (sodium metabisulfite) 100 g, 500 g
for analysis EMSURE® ACS,Reag. Ph Eur

Cat. No. 109057 Hydrochloric acid 1 mol/l Titripur® 1 l, 2.5 l

Alternatively:

Cat. No. 101647 Alcian blue solution 500 ml
for microscopy

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.

Deparaffinize and rehydrate sections in the conventional manner.

Reagent preparation

Staining can be carried out using either a ready-to-use (Cat. No. 101647) or a separately prepared Alcian blue solution (prepared from Cat. No. 105234).

Acetic acid 3 %

Carefully mix 485 ml of distilled water with 15 ml of acetic acid 100 %.

Alcian blue solution

Dissolve 5 g of Alcian blue 8 GX (C.I. 74240) for microscopy Certistain® in 500 ml of acetic acid 3 % with stirring.

The pH is approx. 2.5.

The freshly prepared color solution should be filtered before use.

Alcian blue staining

Procedure

Staining in the staining cell

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

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 histological specimen	
Distilled water	rinse
Alcian blue solution	5 min
Running tap water	3 min
Distilled water	rinse
Nuclear fast red-aluminum sulfate solution 0.1%	10 min
Running tap water	3 min
Distilled water	rinse
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 clearing with xylene or Neo-Clear®, histological samples can be mounted with water-free mounting agents (e.g. Neo-Mount®, Entellan®, DPX new or Entellan® new) and a cover glass and can then be stored.

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

Result

Cell nuclei	red
Acidic mucosubstances	light blue

Alcian blue-PAS staining

Staining can be carried out using e.g. the PAS staining kit, Cat. No. 101646 (contains periodic acid and Schiff's reagent).

Procedure

Staining in the cell

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.

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

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

Slide with histological specimen	
Distilled water	rinse
Alcian blue solution	5 min
Running tap water	3 min
Distilled water	rinse
Reagent 1 (Periodic acid solution)	10 min
Running tap water	3 min
Distilled water	rinse
Reagent 2 (Schiff's reagent)*	15 min
Running tap water	3 min
Distilled water	rinse
Hematoxylin solution modified acc. to Gill III**	20 sec
Running tap water	3 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.	

* To enhance and optimize the specificity of the stain, the tissue should be treated with sulfite water after the reaction with Schiff's reagent (3 x 2 min). Prepare sulfite water by first mixing 10 ml of sodium disulfite solution (10 %) and 10 ml of hydrochloric acid (1 mol/l), and then mixing this solution with 200 ml of tapwater.

** To further enhance the brilliance and contrast of the PAS-positive structures, it is recommended to use hematoxylin solution modified according to Gill II (Cat. No. 105175).

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

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

Result

Cell nuclei	blue
Acidic mucosubstances	light blue
Polysaccharides, neutral mucopolysaccharides	purple

Technical notes

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

When using histoprocessors and 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 (e.g. ISOSLIDE® Alcian blue, Cat. No. 1.00425.0001) should be conducted with each application in order to avoid an incorrect result.

Storage

Store Alcian blue 8 GX (C.I. 74240) for microscopy Certistain® at +5°C to +30°C.

Shelf-life

Alcian blue 8 GX (C.I. 74240) 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.

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.	100063	Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l
Cat. No.	100121	Nuclear fast red-aluminium sulfate solution 0.1% for microscopy	500 ml
Cat. No.	100425	ISOSLIDE® Alcian blue Control Slides with reference tissue for the detection of acid mucosubstances in histological tissue	25 tests
Cat. No.	100496	Formaldehyde solution 4%, buffered, pH 6.9 (approx. 10% Formalin solution) for histology	350 ml and 700 ml (in wide neck bottle), 5 l, 10 l, 10 l Tritripac®
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.	100974	Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 l, 2.5 l
Cat. No.	101646	PAS staining kit for detection of aldehyde and mucosubstances	2x 500 ml
Cat. No.	101647	Alcian blue solution for microscopy	500 ml
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.	105174	Hematoxylin solution modified acc. to Gill III for microscopy	500 ml, 1 l, 2.5 l
Cat. No.	105175	Hematoxylin solution modified acc. to Gill II for microscopy	500 ml, 2.5 l
Cat. No.	106528	Sodium disulfite (sodium metabisulfite) for analysis EMSURE® ACS,Reag. Ph Eur	100 g, 500 g
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.	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.	109057	Hydrochloric acid 1 mol/l Titripur®	1 l, 2.5 l
Cat. No.	109843	Neo-Clear® (xylene substitute) for microscopy	5 l
Cat. No.	111609	Histosec® pastilles solidification 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) solidification point 56-58°C embedding agent for histology	10 kg (4x 2.5 kg), 25 kg

Hazard classification

Cat. No. 105234

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. 105234

C.I. 74240

$C_{56}H_{68}Cl_4CuN_{16}S_4$

M = 1298.88 g/mol

Other IVD products

Cat. No.	100408	ISOSLIDE® PAS Control Slides with reference tissue for the detection of polysaccharides in histological tissue	25 tests
Cat. No.	100524	Periodic acid for analysis EMSURE®	25 g, 100 g
Cat. No.	100820	Methenamine silver plating kit acc. to Gomori for the detection of argent-affine structures in histological tissue	1 set
Cat. No.	102439	Eosin Y-solution 0.5%, alcoholic for microscopy	500 ml, 2.5 l
Cat. No.	102480	Eosin-Phloxine solution, alcoholic for microscopy	500 ml, 1 l
Cat. No.	107509	Pararosaniline (chloride) (C.I. 42500) for microscopy Certistain®	25 g, 100 g
Cat. No.	109033	Schiff's reagent for microscopy	500 ml, 2.5 l
Cat. No.	109149	Mayer's hemalum solution for microscopy	500 ml, 1 l, 2.5 l
Cat. No.	109204	Giemsa's azur eosin methylene blue solution for microscopy	100 ml, 500 ml, 1 l, 2.5 l
Cat. No.	109844	Eosin Y-solution 0.5% aqueous for microscopy	1 l, 2.5 l
Cat. No.	117081	Eosin Y solution 1%, alcoholic for microscopy	1 l

Literature

1. Romeis - Mikroskopische Technik, Editors: Mulisch, Maria, Welsch, Ulrich, 2015, Springer-Verlag Berlin Heidelberg
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



Consult instructions
for use



Manufacturer



Catalog number



Batch code



Caution, consult
accompanying documents



Use by
YYYY-MM-DD



Temperature
limitation

Status: 2017-09-22

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