

1.01647.0500

## Microscopy

# Alcian blue solution, pH 2.5

for microscopy

### For professional use only

 In Vitro Diagnostic Medical Device


### Intended purpose

Alcian blue is used to stain acidic mucopolysaccharides.

This "Alcian blue solution, pH 2.5 - for microscopy" is used for human-medical cell diagnosis and serves the histological investigation of sample material of human origin. It is a ready-to-use staining solution that when used together with other in vitro diagnostic products from our portfolio makes target structures evaluable for diagnostic purposes (by fixing, embedding, staining, counterstaining, mounting) in human-histological specimen materials, for example histological sections of e.g. the intestine or stomach.

The present ready-to-use Alcian blue solution, pH 2.5 can be used for the staining of acid mucopolysaccharides in histological tissue.

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 examinations may be necessary to reach a definitive diagnosis.

### Principle

Acidic mucosubstances are selectively stained in the tissue, after which the specimen is counterstained with nuclear fast red. 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. Counterstaining takes place with a nuclear fast red/aluminium sulfate solution.

The alcian blue staining method is frequently combined with the Periodic acid-Schiff (PAS) reaction. In combination with the PAS reaction, non-substituted polysaccharides, neutral mucopolysaccharides, muco- and glycoproteins and glycol- and phospholipids can be visualized in addition.

### Sample material

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

### Reagents

Cat. No. 1.01647.0500  
Alcian blue solution, pH 2.5  
for microscopy 500 ml

### Also required:

#### 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 (see "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)  
for analysis EMSURE® ACS, Reag. Ph Eur 100 g, 500 g

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

### 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.

When using the corresponding auxiliary reagents, the corresponding instructions for use must be observed.

Deparaffinize and rehydrate sections in the conventional manner.

### Reagent preparation

The Alcian blue solution, pH 2.5 - for microscopy used for staining is ready-to-use, dilution of the solution is not necessary and merely produces a deterioration of the staining result and its stability.

## 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 in order to guarantee an optimal staining result.

Slide with histological specimen	
Distilled water	rinse
Alcian blue solution, pH 2.5	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 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.

### Result

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 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 guarantee an optimal staining result.

Slide with histological specimen	
Distilled water	rinse
Alcian blue solution, pH 2.5	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
Dehydrate with ascending alcohol series and xylene or Neo-Clear® in the conventional manner	
Mount the Neo-Clear®-wet slides with Neo-Mount® or the xylene-wet slides with e.g. Entellan® new and cover glass.	

\* As a measure to avoid a possible tissue-dependent pseudoreaction, the specimens can be treated with sulfite water (3 x 2 min) after the periodic acid incubation procedure.  
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 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.

## Result

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.

## 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 (e.g. ISOSLIDE® Alcian blue, pH 2,5, Cat. No. 1.00425.0001) should be conducted with each application in order to avoid an incorrect result.

## Storage

Store the Alcian blue solution, pH 2,5 - for microscopy at +15 °C to +25 °C.

## Shelf-life

The Alcian blue solution, pH 2,5 - for microscopy can be used up to 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

2000 - 3000 stainings / 500 ml

## 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](http://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. 100121	Nuclear fast red-aluminium sulfate solution 0.1% for microscopy	500 ml
Cat. No. 100425	ISOSLIDE® Alcian blue, pH 2,5 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 bottle with wide neck), 5 l, 10 l, 10 l Titripac®
Cat. No. 100524	Periodic acid for analysis EMSURE®	25 g, 100 g
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. 103699	Immersion oil Type N acc. to ISO 8036 for microscopy	100-ml dropping bottle
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. 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. 109033	Schiff's reagent for microscopy	500 ml, 2.5 l
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. 1.01647.0500

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. 1.01647.0500

C.I. 74240 10 g/l  
pH approx. 2.5

## 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. 100820	Methenamine silver plating kit acc. to Gomori for the detection of argent-affine structures in histological tissue	1 set
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

## 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

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3. Histotechnik, Gudrun Lang, 2013 Springer Verlag, 2. Auflage
4. Theory and Practice of Histological Techniques, John D Bancroft, Marilyn Gamble, 2008, Churchill Livingstone ELSEVIER, 6th Edition
5. Laboratory Manual of Histochemistry, Linda L. Vacca, 1985, Raven Press
6. Histological & Histochemical Methods: Theory & Practice, J. A. Kiernan, 1990, Pergamon Press, 2nd Edition
7. Histological and Histochemical Methods, Theory and practice, J. A. Kiernan, 2015, Scion Publishing Ltd, 5th Edition
8. Conn's Biological Stains, R.W. Horobin, J.A. Kiernan, 2002, Biological Stain Commission Publication, 10th 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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