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## Microscopy

# Pararosaniline (chloride) (C.I. 42500)

for microscopy Certistain®



In Vitro Diagnostic Medical Device



for detection of aldehyde and mucosubstances

This staining dye "Pararosaniline (chloride) (C.I. 42500) - 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 evaluable for diagnostic purposes (by fixing, embedding where necessary, staining with the above pararosaniline solution, counterstaining, mounting).

This Pararosaniline (chloride) (C.I. 42500) Certistain® solid dye is used to prepare the Schiff's reagent required for the PAS reaction, for the staining of mucopolysaccharides in histological tissue specimens.

### Principle

The PAS (periodic acid Schiff) reaction is one of the most frequently used chemical methods for histology.

In the PAS reaction, the histological specimen material is first treated with periodic acid, resulting in the oxidation of the 1,2-glycols into aldehyde groups. The addition of Schiff's reagent (fuchsin-sulfuric acid) in the second step causes the aldehydes to react to form a brilliant red color. In the end result, the PAS reaction yields a specific color reaction with unsubstituted polysaccharides, neutral mucopolysaccharides, muco- and glycoproteins, and glyco- and phospholipids.

The PAS reaction can be further combined with the Alcian blue staining method to detect mucosubstances (glycosaminoglycans).

### Sample material

Sections of formalin fixed, paraffin embedded tissue (3 - 4 µm thick paraffin sections) or cell smears are used as starting material.

### Reagents

Cat. No. 107509

Pararosaniline (chloride) (C.I. 42500) 25 g, 100 g  
for microscopy Certistain®

Color Index No.: 42500

Color Index Name: Basic red 9

### Also required (PAS staining):

Cat. No. 102186 Charcoal activated for analysis 250 g, 1 kg

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

Cat. No. 106597 Sodium metaperiodate for analysis 50 g, 250 g  
EMSURE® ACS, Reag. Ph Eur

or

Cat. No. 100524 Periodic acid for analysis EMSURE® 25 g, 100 g

### Optional (see "PAS staining - Procedure", footnotes):

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

### Alternatively:

Cat. No. 109033 Schiff's reagent for microscopy 500 ml, 2.5 l

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

### 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

#### Schiff's reagent

The stain can be carried out either with a ready-to-use (Cat. No. 109033) or with a separately prepared Schiff's reagent (from Cat. No. 107509).

Dissolve 0.5 g of Pararosaniline (chloride) (C.I. 42500) Certistain® in 15 ml of hydrochloric acid (1 mol/l).

Dissolve 0.5 g of sodium disulfite in 85 ml of distilled water.

Mix the two solutions and leave to stand at room temperature for 24 hours.

Add 0.3 g of activated charcoal, shake vigorously for 15 sec.

The solution initially has a tender pink tinge, but soon becomes colorless.

The freshly prepared color solution should be filtered before use.

Store the solution refrigerated in a brown bottle.

#### Periodic acid solution:

The stain can be carried out either with the ready-to-use solution provided in the PAS staining kit (Cat. No. 101646) or with a separately prepared periodic acid solution (e.g. from Cat. No. 106597 or Cat. No. 100524).

Dissolve 0.8 g of sodium metaperiodate / periodic acid in 100 ml of distilled water.

## PAS 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
Periodic acid solution	5 min
Running tap water	3 min
Distilled water	rinse
Schiff's reagent*	15 min
Running tap water	3 min
Distilled water	rinse
Hematoxylin solution modified acc. to Gill III**	2 min
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 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

Nuclei blue  
Polysaccharides, glycogen, neutral mucopolysaccharides, muco- and glycoproteins, glyco- and phospholipids, basal membrane, collagen purple

## Alcian blue PAS staining

For more precise information on the procedure and other details refer to the package inserts for

PAS staining kit, Cat. No. 101646 or

Alcian blue solution, Cat No. 101647 or

Alcian blue 8 GX (C.I. 74240) Certistain®, Cat. No. 105234.

## Technical notes

The freshly prepared color solution should be filtered before use.

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 trained personnel.

Valid nomenclatures must be used.

Further tests must be selected and implemented according to recognized methods.

Suitable controls (e.g. ISOSLIDE® PAS, Cat. No. 1.00408.0001, ISOSLIDE® Alcian blue, Cat. No. 1.00425.0001) should be conducted with each application in order to avoid an incorrect result.

## Storage

Store Pararosaniline (chloride) (C.I. 42500) - for microscopy Certistain® at +5 °C to +30 °C.

Due to the light-sensitivity of Schiff's reagent, the storage should preferably be performed in the dark.

## Shelf-life

Pararosaniline (chloride) (C.I. 42500) - 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](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.	100408	ISOSLIDE® PAS Control Slides with reference tissue for the detection of polysaccharides in histological tissue	25 tests
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 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.	101647	Alcian blue solution for microscopy	500 ml
Cat. No.	102572	Schiff's reagent Intense - for the detection of aldehyde and mucosubstances in microscopy	1 l
Cat. No.	103693	Cryoembedding media for microscopy M-FREEZE™	100 ml
Cat. No.	103699	Immersion oil 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.	105234	Alcian blue 8 GX (C.I.74240) for microscopy Certistain®	10 g
Cat. No.	106528	Sodium disulfite (sodium metabisulfite) for analysis EMSURE® ACS, Reag. Ph Eur	100 g, 500 g
Cat. No.	106597	Sodium metaperiodate for analysis EMSURE® ACS, Reag. Ph Eur	50 g, 250 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.	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. 107509

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. 107509

C.I. 42500

C<sub>19</sub>H<sub>18</sub>ClN<sub>3</sub>

M = 323.83 g/mol

## 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: 2019-09-20

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