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## Microscopy

### Erythrosine B (C.I. 45430)

for microscopy Certistain®

 In Vitro Diagnostic Medical Device


for counterstaining in hematoxylin and eosin staining (H&E) in histology and clinical cytology

This staining dye "Erythrosine B (C.I. 45430) - 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 evaluable for diagnostic purposes (by fixing, embedding, staining with the above erythrosine B solution, counterstaining, mounting) in histological specimen materials, for example histological sections of e.g. the kidney, muscle tissue, heart, or lung.

The hematoxylin and eosin (H&E) staining method is the method most frequently used for the staining of histology material.

#### Principle

The H&E stain is an overview stain. The staining mechanism behind the H&E staining is a physico-chemical process.

In the first step, the positively charged nuclear dye (hematoxylin) binds to the negatively charged phosphate groups of the nucleic acid of the cell nucleus.

The nuclei will be dyed dark blue to dark violet.

The second step is the counterstaining with negatively charged anionic Erythrosine B, an xanthene dye. Erythrosine binds to the positively charged plasma proteins.

Cytoplasm and intercellular substances are stained pink to red, while erythrocytes will appear with yellow to orange color.

#### Sample material

Sections of formalin fixed, paraffin embedded tissue (3 - 4 µm thick paraffin sections) or cryo sections, as well as clinico-cytological specimens material are used as starting material.

#### Reagents

Cat. No. 115934

Erythrosine B (C.I. 45430) 10 g, 25 g, 1 kg  
for microscopy Certistain®  
Color Index No.: 45430  
Color Index Name: Acid red 51, Food red 14

#### Also required:

Cat. No. 109249 Mayer's hemalum solution 500 ml, 1 l, 2.5 l  
for microscopy

or

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

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

Cat. No. 100316 Hydrochloric acid 25% 1 l, 2.5 l  
for analysis EMSURE®

#### 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

##### Erythrosine B-solution, 0.5 %, aqueous, working solution

For preparation of approx. 1000 ml solution mix:

Erythrosine B (C.I. 45430) Certistain®	5.0 g
Distilled water	1000 ml
dissolve	
Acetic acid 100 %	2.0 ml
add and mix	

The freshly prepared staining solution should be filtered before use.

##### Hydrochloric acid 0.1 %, aqueous

For preparation of approx. 100 ml solution mix:

Hydrochloric acid 25%	0.4 ml
Distilled water	100 ml

## H&E staining

### Procedure of staining paraffin sections

#### 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 paraffin section	
Distilled water	1 min
Mayer's hemalum solution or Hematoxylin solution modified acc. to Gill III	3 min
Hydrochloric acid 0.1 %, aqueous	2 sec
Running tap water	3 - 5 min
Erythrosine B-solution 0.5 %, aqueous, working solution	3 min
Running tap water	30 sec
Ethanol 70 %	3 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. DPX new, Entellan® new, Neo-Mount®) 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	dark blue to dark violet
Cytoplasm, intercellular substances	pink to red
Erythrocytes	yellow to orange

#### 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.

## Diagnosics

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 Erythrosine B (C.I. 45430) - for microscopy Certistain® at +5 °C to +30 °C.

## Shelf-life

Erythrosine B (C.I. 45430) - 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. 100063	Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l
Cat. No. 100316	Hydrochloric acid 25% for analysis EMSURE®	1 l, 2.5 l
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. 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. 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. 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. 109249	Mayer's hemalum solution for microscopy	500 ml, 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. 115936

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. 115936  
C.I. 45430  
 $C_{20}H_{16}I_4Na_2O_5$   
M = 879.86 g/mol

## Other IVD products

Cat. No. 101646	PAS staining kit for detection of aldehyde and mucosubstances	2x 500 ml
Cat. No. 101728	OSTEOSOFT® mild decalcifier-solution for histology	1 l, 10 l Titripac®
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. 102572	Schiff's reagent Intense - for the detection of aldehyde and mucosubstances in microscopy	1 l
Cat. No. 105175	Hematoxylin solution modified acc. to Gill II for microscopy	500 ml, 2.5 l
Cat. No. 109033	Schiff's reagent for microscopy	500 ml, 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: 2019-09-27

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