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## Microscopy

### Eosin Y (yellowish) (C.I. 45380)

for microscopy Certistain®

#### For professional use only

IVD

In Vitro Diagnostic Medical Device



#### Intended purpose

This staining dye "Eosin Y (yellowish) (C.I.45380) - 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 Eosin Y 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.

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 tests must be carried out according to recognized, valid methods to reach a definitive diagnosis.

#### 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 second step is the counterstaining with negatively charged anionic Eosin Y, a xanthene dye. Eosin binds to the positively charged plasma proteins.

#### Sample material

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

#### Reagents

Cat. No. 115935

Eosin Y (yellowish) (C.I. 45380) 25 g, 100 g, 1 kg

for microscopy Certistain®

Color Index No.: 45380

Color Index Name: Acid red 87, Pigment red 90

#### 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. 500 ml, 1 l, 2.5 l  
to Gill III  
for microscopyCat. No. 100063 Acetic acid (glacial) 100% anhydrous 1 l, 2.5 l  
for analysis EMSURE® ACS,ISO,Reag. Ph EurCat. No. 100316 Hydrochloric acid 25% 1 l, 2.5 l  
for analysis EMSURE®

#### Alternatively:

Cat. No. 102439 Eosin Y-solution 0.5%, alcoholic 500 ml, 2.5 l  
for microscopyCat. No. 109844 Eosin Y-solution 0.5% aqueous 1 l, 2.5 l  
for microscopyCat. No. 117081 Eosin Y solution 1%, alcoholic 1 l  
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.

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

Deparaffinize and rehydrate sections in the conventional manner.

#### Reagent preparation

Staining can be carried out using either a ready-to-use (Cat. Nos. 102439, 109844, or 117081) or a separately prepared eosin Y solution (prepared from Cat. No. 115935).

#### Eosin Y-solution, 0.5 %, aqueous, working solution

For preparation of approx. 500 ml solution mix:

Eosin Y (yellowish) (C.I.45380) Certistain®	2.5 g
Distilled water	500 ml
dissolve	
Acetic acid 100 %	1.0 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 in order 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
Eosin Y-solution 0.5 %, aqueous, working solution	3 min
Running tap water	30 sec
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. 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

Cell nuclei	dark blue to dark violet
Cytoplasm, intercellular substances	pink to red
Erythrocytes	yellow to orange

## Trouble-shooting

### Weak staining of cytoplasm and connective tissue structures

For the intensification of the eosin staining, an acidified working solution (using e.g. glacial acetic acid) should be used. The use of a non-acidified solution will result in weakly stained cytoplasm and connective tissue structures, hence it is advisable to follow the specified Reagent preparation protocol to achieve an optimal staining result.

## Technical notes

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

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 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 should be conducted with each application in order to avoid an incorrect result.

## Storage

Store Eosin Y (yellowish) (C.I.45380) - for microscopy Certistain® at +5 °C to +30 °C.

## Shelf-life

Eosin Y (yellowish) (C.I.45380) - 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. 102439	Eosin Y-solution 0.5%, alcoholic for microscopy	500 ml, 2.5 l
Cat. No. 103693	M-FREEZE™ Cryoembedding media for microscopy	100 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. 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. 109844	Eosin Y-solution 0.5% aqueous for microscopy	1 l, 2.5 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
Cat. No. 117081	Eosin Y solution 1%, alcoholic for microscopy	1 l

## Hazard classification

Cat. No. 115935

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. 115935  
C.I. 45380  
 $C_{20}H_6Br_4Na_2O_5$   
M = 691.86 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. 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

## 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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2. Theory and Practice of Histological Techniques, John D Bancroft, Marilyn Gamble, 2008, Churchill Livingstone ELSEVIER, sixth 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
4. Basiswissen Histologie und Zytologie, Karl Heinz Stein, Hellmut Flenker, 3. Auflage 2004
5. Histological and Histochemical Methods, Theory and practice, J. A. Kiernan, 2015, Scion Publishing Ltd, 5th Edition
6. Histotechnik, Gudrun Lang, 2013 Springer Verlag, 2. Auflage
7. Laboratory Manual of Histochemistry, Linda L. Vacca, 1985, Raven Press
8. Staining Procedures, George Clark, 1981, Williams & Wilkins, fourth Edition
9. Welsch Sobotta - Lehrbuch Histologie, Editor: Ulrich Welsch, 2006, ELSEVIER Urban&Fischer, 2. Auflage



Consult instructions  
for use



Manufacturer



Catalog number



Batch code



Caution, consult  
accompanying documents



Use by  
YYYY-MM-DD



Temperature  
limitation

Status: 2021-Jun-29

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