Sigma-Aldrich®

1.15941.0025 1.15941.0100 1.15941.1000

Microscopy

Light green SF yellowish (C.I. 42095)

for microscopy Certistain®

For professional use only



In Vitro Diagnostic Medical Device



Intended purpose

This staining dye "Light green SF yellowish (C.I. 42095) - for microscopy Certistain®" is used for human-medical cell diagnosis and serves the purpose of the histological and cytological 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 where necessary, staining with the above light green SF solution, counterstaining, mounting) in histological and cytological specimen materials.

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.

This staining solution, which requires further preparation before it can be used, serves exclusively for overview staining in the differentiation of the target structures of cell and tissue components listed below in the section "Result". For more specific applications we recommend the use of Cat. No. 109271 Papanicolaou's solution 3a polychromatic solution EA 31 for cytology or Cat. No. 109272 Papanicolaou's solution 3b polychromatic solution EA 50 for cytology.

Principle

Light green SF yellowish is used for routine staining methods in histology, e.g. the staining of collagen fibers and especially for Masson's trichrome staining acc. to Goldner.

Employing a combination of three different staining solutions, the trichrome staining procedure is used to selectively visualize muscle fibers, collagen fibers, fibrin, and erythrocytes.

The original methods were primarily used for the differentiation of collagen and muscle fibers. The selection of the dyes used, which differ in their molecule size, yields a differentiated staining result for the individual tissue components.

Sample material

Starting materials are sections of tissue embedded in paraffin (3 - 5 μm thick paraffin sections).

Reagents

3	
Cat. No. 115941	
Light green SF yellowish (C.I. 42095)	25 g, 100 g,
for microscopy Certistain®	1 kg
Color Index No.: 42095	
Color Index Name: Acid green 5, Food green 2	

Also required:

Cat. No. 100063 Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l
Cat. No. 100532 Molybdatophophoric acid hydrate for analysis EMSURE® ACS,Reag. Ph Eur	25 g, 100 g
Cat. No. 104185 Orange G (C.I. 16230) for microscopy Certistain®	25 g
Cat. No. 104190 Fuchsin acid (C.I. 42685) for microscopy Certistain®	25 g
Cat. No. 104238 Ponceau S (C.I. 27195) for microscopy Certistain®	25 g
Cat. No. 115973 Weigert's iron hematoxylin kit for nuclear staining in histology	2x 500 ml

Alternatively:

Instead of the combination of single reagents, the staining kit 1.00485.0001 can be used:

Cat. No. 1.00485.0001 Masson-Goldner staining kit

1 unit

for the visualization of connective tissue with trichromic staining

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

Weigert's iron hematoxylin staining solution

Mix Weigert's solution A and Weigert's solution B (from Cat. No. 115973) in the ratio $1\,+\,1.$

The prepared staining solution remains stable for approx. one working week. The solution must be exchanged as soon as the cell nuclei have been stained brown.

Ponceau S / fuchsin acid solution

For preparation of approx. 300 ml solution mix:

Ponceau S (C.I. 27195) Certistain®	0.2 g
Fuchsin acid (C.I. 42685) Certistain®	0.1 g
,	+
Distilled water	300 ml
Acetic acid 100 %	0.6 ml
dissolve and filter	

Orange G solution

For preparation of approx. 100 ml solution mix:

Orange G (C.I. 16230) Certistain®	2 g
Molybdatophophoric acid hydrate	5 g
Distilled water	100 ml
dissolve and filter	

Light green SF solution

For preparation of approx. 100 ml solution mix:

Light green SF yellowish (C.I. 42095) Certistain®	0.2 g
Distilled water	100 ml
Acetic acid 100 %	0.2 ml
dissolve and filter	

Acetic acid 1 %

For preparation of approx. 1000 ml solution mix:

Acetic acid 100 %	10 ml
Distilled water	990 ml

The freshly prepared staining solutions should be filtered before use.

Procedure

Trichrome staining in the staining cell

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

The slides must be immersed and moved about in the solutions, simple immersion alone yields inadequate staining results.

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.

lide with histological specimen		
Distilled water	1 min	
Weigert's iron hematoxylin staining solution	3 min	
Running tap water	3 - 5 min	
Ponceau S / fuchsin acid solution	3 min	
Acetic acid 1 %	rinse briefly	
Orange G solution	25 sec	
Acetic acid 1 %	rinse briefly	
Light green SF solution	5 min	
Acetic acid 1 %	rinse briefly	
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 slides can be covered with non-aqueous mounting agents (e.g. 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 brown to black
Cytoplasm, muscle brick red
Connective tissue, acid mucous substances
Erythrocytes green
bright orange

Trouble-shooting

When the specimen is incubated in ethanol in ascending alcohol concentration for too long a time, the Light green SF staining dye may be washed out of e.g. the connective-tissue structures. It is hence advisable to observe the specified times to achieve an optimal result.

Technical notes

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

The freshly prepared staining solutions 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 Light green SF yellowish (C.I. 42095) - for microscopy Certistain $^{\rm g}$ at +5 $^{\rm o}$ C to +30 $^{\rm o}$ C.

Shelf-life

Light green SF yellowish (C.I. 42095) - 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 $+5\,^{\circ}\text{C}$ to $+30\,^{\circ}\text{C}.$

The bottles must be kept tightly closed at all times.

If stored at $\pm 15^{\circ}$ C to $\pm 25^{\circ}$ C, the freshly prepared Weigert's iron hematoxylin staining solution can be used for minimum one working week. The solution must be exchanged as soon as the cell nuclei have been stained brown.

However, the solutions should be discarded when contaminations (e.g. fungi, bacteria), that occur at times, are observed.

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.

Üsed 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

Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l
Masson-Goldner staining kit for the visualization of connective tissue with trichromic staining	1 unit
Molybdatophophoric acid hydrate for analysis EMSURE® ACS,Reag. Ph Eur	25 g, 100 g
DPX new non-aqueous mounting medium for microscopy	500 ml
Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 I, 2.5 I
Immersion oil Type N acc. to ISO 8036 for microscopy	100-ml drop- ping bottle
Orange G (C.I. 16230) for microscopy Certistain®	25 g
Fuchsin acid (C.I. 42685) for microscopy Certistain®	25 g
Ponceau S (C.I. 27195) for microscopy Certistain®	25 g
Immersion oil for microscopy	100-ml drop- ping bottle, 100 ml, 500 ml
Entellan® rapid mounting medium for microscopy	500 ml
Entellan® new rapid mounting medium for microscopy	100 ml, 500 ml, 1 l
Xylene (isomeric mixture) for histology	4
Neo-Mount® anhydrous mounting medium for microscopy	100-ml drop- ping bottle, 500 ml
Neo-Clear® (xylene substitute) for microscopy	5 I
Weigert's iron hematoxylin kit for nuclear staining in histology	2x 500 ml
	ACS,ISÓ,Reag. Ph Eur Masson-Goldner staining kit for the visualization of connective tissue with trichromic staining Molybdatophophoric acid hydrate for analysis EMSURE® ACS,Reag. Ph Eur DPX new non-aqueous mounting medium for microscopy Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE® Immersion oil Type N acc. to ISO 8036 for microscopy Orange G (C.I. 16230) for microscopy Certistain® Fuchsin acid (C.I. 42685) for microscopy Certistain® Ponceau S (C.I. 27195) for microscopy Certistain® Immersion oil for microscopy Entellan® rapid mounting medium for microscopy Sentellan® rapid mounting medium for microscopy Xylene (isomeric mixture) for histology Neo-Mount® anhydrous mounting medium for microscopy Neo-Clear® (xylene substitute) for microscopy Weigert's iron hematoxylin kit

Hazard classification

Cat. No. 115941

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 product components

Cat. No. 115941 C.I. 42095 $C_{37}H_{34}N_2Na_2O_9S_3$ M = 792.86 g/mol

Other IVD products

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.	101287	Löffler's methylene blue solution for microscopy	100 ml, 500 ml, 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.	109271	Papanicolaou's solution 3a polychromatic solution EA 31 for cytology	500 ml, 2.5 l
Cat. No.	109272	Papanicolaou's solution 3b polychromatic solution EA 50 for cytology	500 ml, 1 l, 2.5 l

Cat. No. 113741 Lactophenol blue solution 100 ml for staining fungi Cat. No. 115161 Histosec® pastilles (without DMSO) solidification point 56-58°C 10 kg (4x 2.5 kg), 25 kg embedding agent for histology 1 set

Cat. No. 116450 AFB-Color staining kit for the microscopic investigation of

acid-fast bacteria (AFB) (cold staining)

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

- 1. Routine Cytological Staining Techniques: Theoretical Background and Practice, Mathilde E. Boon, Johanna S. Drijver, 1986, Elsevier Science Publishing Company
- 2. Romeis Mikroskopische Technik, Editors: Maria Mulisch, Ulrich Welsch, 2015, Springer Spektrum, 19. Auflage
- 3. Theory and Practice of Histological Techniques, John D Bancroft, Marilyn Gamble, 2008, Churchill Livingstone ELSEVIER, sixth Edition
- 4. 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
- 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



Manufacturer





Caution, consult accompanying documents



YYYY-MM-DD



Temperature limitation

Status: 2021-Jun-21

Merck KGaA, 64271 Darmstadt, Germany, Tel. +49(0)6151 72-2440

www.microscopy-products.com
EMD Millipore Corporation, 400 Summit Drive
Burlington MA 01803, USA, Tel. +1-978-715-4321 Sigma-Aldrich Canada Co. or Millipore (Canada) Ltd. 2149 Winston Park, Dr. Oakville, Ontario, L6H 6J8 Phone: +1 800-565-1400

