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# Microscopy

# Methyl green zinc chloride double salt (C.I. 42590)

for microscopy Certistain®

IVD In Vitro Diagnostic Medical Device

CE

This staining dye “Methyl green zinc chloride double salt (C.I. 42590) - 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 (by fixing, where necessary embedding, staining with the above methyl green solution, counterstaining, mounting) evaluable for diagnostic purposes.

## Principle

Methyl green belongs to the group of triphenylmethane dyes.

Methyl green is used together with pyronine G in a one-step method for staining of DNA (green) and RNA (red).
Methyl green is also used as a counterstain in other reactions, such as demonstration of enzyme activity with diazonium salt methods.

## Sample material

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

## Reagents

Cat. No. 115944

Methyl green zinc chloride double salt (C.I. 42590) for microscopy Certistain® — 25 g, 1 kg

Color Index No.: 42590
Color Index Name: -

**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. | 100983 | Ethanol absolute for analysis EMSURE® ACS,ISO,Reag. Ph Eur | 1 l, 2.5 l, 5 l |
| Cat. No. | 101990 | 1-Butanol for analysis EMSURE® ACS,ISO,Reag. Ph Eur | 1 l, 2.5 l, 4 l, 10 l |
| Cat. No. | 102445 | Chloroform for analysis EMSURE® ACS,ISO,Reag. Ph Eur | 1 l, 2.5 l, 4 l, 10 l |
| Cat. No. | 106268 | Sodium acetate anhydrous for analysis EMSURE® ACS,Reag. Ph Eur | 250 g, 1 kg, 2.5 kg |
| Cat. No. | 107518 | Pyronine G (C.I. 45005) for microscopy Certistain® | 25 g |
| Cat. No. | 109944 | Acetic acid for 1000 ml, c($CH_3COOH$) = 0.1 mol/l (0.1 N) Titrisol® | 1 amp |

## Sample prepration

The sampling must be performed by qualified personnel.

**Fixation**

Fix histological slides in the conventional manner with Carnoy’s fixing solution.

For preparation of Carnoy’s fixing solution mix:

| | |
|---|---|
| Ethanol absolute | 6 parts |
| Chloroform | 3 parts |
| Acetic acid 100 % | 1 part |
| mix | |

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.

## Reagent preparation

**Acetate buffer solution pH 4.8**

Sodium acetate solution 0.1 N

For preparation of approx. 1000 ml solution mix:

| | |
|---|---|
| Sodium acetate anhydrous | 8.20 g |
| Distilled water | 1000 ml |
| dissolve | |

Acetate buffer solution pH 4.8

For preparation of approx. 100 ml solution mix:

| | |
|---|---|
| Sodium acetate solution 0.1 N | 56.6 ml |
| Acetic acid c($CH_3COOH$) = 0.1 mol/l (0.1 N) Titrisol® | 43.4 ml |
| mix | |

**Methyl green solution**

For preparation of approx. 100 ml solution mix:

| | |
|---|---|
| Methyl green zinc chloride double salt (C.I. 42590) Certistain® | 2 g |
| Distilled water (warm) | 100 ml |
| dissolve | |
| extract with chloroform and discard the organic phase | |
| filter the aqueous staining solution | |

**Pyronine G solution**

For preparation of approx. 100 ml solution mix:

| | |
|---|---|
| Pyronine G (C.I. 45005) Certistain® | 5 g |
| Distilled water | 100 ml |
| dissolve | |
| extract with chloroform and discard the organic phase | |
| filter the aqueous staining solution | |

The freshly prepared staining solutions should be filtered before use.

**Methyl green-pyronine G solution**

For preparation of approx. 280 ml solution mix:

| | |
|---|---|
| Methyl green solution | 17.5 ml |
| Pyronine G solution | 10 ml |
| Distilled water | 250 ml |
| mix | |

**Methyl green-pyronine G working solution**

For preparation of approx. 100 ml solution mix:

| | |
|---|---|
| Methyl green-pyronine G solution | 50 ml |
| Acetate buffer solution pH 4.8 | 50 ml |
| mix | |

## Procedure

**DNA/RNA staining**

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 | rinse |
| press in filter paper | |
| Methyl green-pyronine G working solution | 20 - 30 min |
| Acetate buffer solution pH 4.8 | rinse |
| press in filter paper | |
| 1-Butanol | 5 min |
| 1-Butanol | 5 min |
| Xylene | 5 min |
| Mount the xylene-wet slides with e.g. Entellan® new and cover glass. | |

After dehydration (ascending alcohol series) and clearing with xylene, histological samples can be mounted with water-free mounting agents (e.g. DPX new or Entellan® new) and a cover glass and and can then be stored.

The use of immersion oil is recommended for the analysis of stained slides with a microscopic magnification >40x.

## Result

| | |
|---|---|
| Chromatin | blue-green |
| Cell nuclei | red |
| Cytoplasm | pink to red |

## 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.
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 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 Methyl green zinc chloride double salt (C.I. 42590) - for microscopy Certistain® at +5 °C to +30 °C.

## Shelf-life

Methyl green zinc chloride double salt (C.I. 42590) - 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. 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. | 100974 | Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE® | 1 l, 2.5 l |
| Cat. No. | 100983 | Ethanol absolute for analysis EMSURE® ACS,ISO,Reag. Ph Eur | 1 l, 2.5 l, 5 l |
| Cat. No. | 101990 | 1-Butanol for analysis EMSURE® ACS,ISO,Reag. Ph Eur | 1 l, 2.5 l, 4 l, 10 l |
| Cat. No. | 102445 | Chloroform for analysis EMSURE® ACS,ISO,Reag. Ph Eur | 1 l, 2.5 l, 4 l, 10 l |
| Cat. No. | 104699 | Immersion oil for microscopy | 100-ml dropping bottle, 100 ml, 500 ml |
| Cat. No. | 106268 | Sodium acetate anhydrous for analysis EMSURE® ACS,Reag. Ph Eur | 250 g, 1 kg, 2.5 kg |
| Cat. No. | 107518 | Pyronine G (C.I. 45005) for microscopy Certistain® | 25 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. | 109944 | Acetic acid for 1000 ml, c($CH_3COOH$) = 0.1 mol/l (0.1 N) Titrisol® | 1 amp |

## Hazard classification

Cat. No. 115944

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. 115944

C.I. 42590
$C_{27}H_{35}BrClN_3 \times ZnCl_2$
M = 653.24 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 wide neck bottle), 5 l, 10 l, 10 l Tritripac® |
| 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 for microscopy | 500 ml, 1 l, 2.5 l |
| Cat. No. | 115161 | Histosec® pastilles (without DMSO) soldification 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 |

## 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: 2017-09-15


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