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1.15933.0025

## Microscopy

### Carmine (C.I. 75470)

(calcium-aluminium lacquer of carminic acid)  
for microscopy Certistain®

**IVD** In Vitro Diagnostic Medical Device



for staining nuclei and for demonstration of glycogen in histological sections  
This staining dye "Carmine (C.I. 75470) (calcium-aluminium lacquer of carminic acid) - 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 carmine solution, counterstaining, mounting) evaluable for diagnostic purposes.

#### Principle

is the calcium-aluminium lacquer of carminic acid  
Carmine is a natural dye (cochineal dye) with a structure that has not been fully elucidated. It is obtained by extraction of scale insects (*Dactylopius coccus cacti*). Carmine is used together with a mordant solution of lithium, aluminium or boron as a lake for staining. Carmine is used for various stainings, e.g. for demonstration of glycogen and mucous substances (Best's carmine), for nuclear staining after silver impregnation or for vital staining.

#### Sample material

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

#### Reagents

Cat. No. 115933  
Carmine (C.I. 75470) 5 g, 25 g  
(calcium-aluminium lacquer of carminic acid)  
for microscopy Certistain®

#### Also required:

##### for nuclear staining

Cat. No. 100327 Hydrochloric acid in ethanol 1 l, 5 l  
for microscopy  
Cat. No. 105680 Lithium carbonate 250 g  
for analysis EMSURE® ACS,ISO,Reag. Ph Eur  
Cat. No. T0501 Thymol ≥99.0% Sigma

##### for demonstration of glycogen

Cat. No. 100974 Ethanol denatured with about 1 l, 2.5 l  
1 % methyl ethyl ketone  
for analysis EMSURE®  
Cat. No. 104928 Potassium carbonate 500 g, 1 kg  
for analysis EMSURE® ACS,ISO,Reag. Ph Eur  
Cat. No. 104936 Potassium chloride 250 g, 500 g,  
for analysis EMSURE® 1 kg  
Cat. No. 105432 Ammonia solution 25% 1 l, 2.5 l, 5 l  
for analysis EMSURE®  
Cat. No. 106009 Methanol 1 l, 2.5 l, 5 l  
for analysis EMSURE® ACS,ISO,Reag. Ph Eur  
Cat. No. 109249 Mayer's hemalum solution 500 ml, 1 l, 2.5 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.  
Deparaffinize and rehydrate sections in the conventional manner.

## Reagent preparation

### Nuclear staining

#### Carmine solution

For preparation of approx. 100 ml solution mix:

Carmine (C.I. 75470) Certistain®	3.6 g
saturated aqueous lithium carbonate solution (approx. 1.25 %)	100 ml
boil for 15 min cool	
Thymol	1 g
add, dissolve, and filter	

### Demonstration of glycogen with Best's carmine solution

#### Best's carmine stock solution

For preparation of approx. 80 ml solution mix:

Carmine (C.I. 75470) Certistain®	2 g
Potassium chloride	5 g
Distilled water	60 ml
dissolve while heating	
Potassium carbonate	1 g
add and <b>carefully</b> bring to the boil (strong foaming!)	
allow to boil for a few minutes, the color changes to dark red cool	
Ammonia solution 25%	20 ml
add and mix	
Close firmly and keep in a cool place. The solution remains stable for about 2 months.	

#### Best's carmine working solution

For preparation of approx. 80 ml solution mix:

filtered carmine stock solution	20 ml
Ammonia solution 25%	30 ml
Methanol	30 ml
mix	

#### Differentiating solution

For preparation of approx. 220 ml solution mix:

Methanol	40 ml
Ethanol	80 ml
mix	
Distilled water	100 ml
add	

The freshly prepared staining solutions should be filtered before use.

### Procedure Nuclear 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	1 min
Carmine solution	3 min
Hydrochloric acid in ethanol ( <b>immediately</b> )	1 min
Hydrochloric acid in ethanol	1 min
Hydrochloric acid in ethanol	1 min
Distilled water	rinse
Ethanol 96 %	1 min
Ethanol 96 %	1 min
Ethanol 100 %	1 min
Ethanol 100 %	1 min
Xylene or Neo-Clear®	1 min
Xylene or Neo-Clear®	1 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 or 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	red
Tissue	light pink, colorless

## Procedure Demonstration of glycogen with Best's carmine solution

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	3 - 5 min
Tap water	3 - 5 min
Best's carmine working solution	5 - 20 min
Differentiating solution (change 1x)	rinse until no further clouds of dye are produced
Ethanol 80 %	rinse
Ethanol 96 %	1 min
Ethanol 96 %	1 min
Ethanol 100 %	1 min
Ethanol 100 %	1 min
Xylene or Neo-Clear®	1 min
Xylene or Neo-Clear®	1 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 or 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

Glycogen	red
Mucous, fibrin, amyloid	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.

Care should be taken that as little ammonia as possible evaporates from the Best's carmine stock solution and working solution.

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 Carmine (C.I. 75470) (calcium-aluminium lacquer of carminic acid) - for microscopy Certistain® at +5 °C to +30 °C.

## Shelf-life

Carmine (C.I. 75470) (calcium-aluminium lacquer of carminic acid) - 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.

Close the Carmin stock solution for the demonstration of glycogen firmly and keep in a cool place. The solution remains stable for about 2 months.

## 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.	100327	Hydrochloric acid in ethanol for microscopy	1 l, 5 l
Cat. No.	100974	Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 l, 2.5 l
Cat. No.	104699	Immersion oil for microscopy	100-ml dropping bottle, 100 ml, 500 ml
Cat. No.	104928	Potassium carbonate for analysis EMSURE® ACS,ISO,Reag. Ph Eur	500 g, 1 kg
Cat. No.	104936	Potassium chloride for analysis EMSURE®	250 g, 500 g, 1 kg
Cat. No.	105432	Ammonia solution 25% for analysis EMSURE®	1 l, 2.5 l, 5 l
Cat. No.	105680	Lithium carbonate for analysis EMSURE® ACS,ISO,Reag. Ph Eur	250 g
Cat. No.	106009	Methanol for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l, 5 l
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.	T0501	Thymol ≥99.0%	Sigma

### Hazard classification

Cat. No. 115933

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. 115933

C.I. 75470

$C_{44}H_{37}O_{27}AlCa \times 3 H_2O$

M = 1118.78 g/mol

### Other IVD products

Cat. No.	100121	Nuclear fast red-aluminium sulfate solution 0.1% for microscopy	500 ml
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.	105175	Hematoxylin solution modified acc. to Gill II 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.	115976	Weigert's iron hematoxylin kit for nuclear staining in histology	2x 500 ml

### 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. Horbin, R.W. and Kiernan, J.A). Bios, 2002



Consult instructions  
for use



Manufacturer



Catalog number



Batch code



Caution, consult  
accompanying documents



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