

1.15931.0025

Microscopy

Acridine orange zinc chloride double salt (C.I.46005)

for microscopy Certistain®



In Vitro Diagnostic Medical Device



This staining dye "Acridine orange zinc chloride double salt (C.I.46005) - for microscopy Certistain®" is used for human-medical cell diagnosis and serves the purpose of the 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 acridine orange solution, counterstaining, mounting) in cytological specimen materials.

Principle

Acridine orange belongs to the group of acridine dyes.

Acridine orange is used as a fluorescence dye for differential staining of DNA and RNA. Many staining procedures involve demonstration of DNA and DNA-rich structures.

Acridine orange is also used to stain microorganisms in unfixed specimens and for vital staining.

Sample material

Fresh, native whole blood or bone-marrow smears as well as clinical cytological material like urine sediment, sputum, smears from fine needle aspiration biopsies (FNAB), rinses, imprints are used as starting material.

Reagents

Cat. No. 1.15931.0025

Acridine orange zinc chloride double salt (C.I.46005) 25 g
for microscopy Certistain®

Color Index No.: 46005

Color Index Name: Basic orange 14

Also required:

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

Cat. No. 102378 Calcium chloride anhydrous powder 500 g, 2.5 kg
Reag. Ph Eur

Cat. No. 104873 Potassium dihydrogen phosphate 250 g, 1 kg
for analysis EMSURE® ISO

Cat. No. 106580 di-Sodium hydrogen phosphate dihydrate 500 g, 1 kg
for analysis EMSURE®

Sample preparation

The sampling must be performed by qualified personnel.

Fixation

Slide with air-dried smear	
Mixture of diethyl ether / ethanol (1 + 1)	15 min

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

Phosphate buffer solution pH 6

Stock solution A - potassium dihydrogen phosphate buffer 0.1 M

For preparation of approx. 1000 ml solution mix:

Potassium dihydrogen phosphate	13.61 g
Distilled water	1000 ml
dissolve	

Stock solution B - di-sodium hydrogen phosphate buffer 0.1 M

For preparation of approx. 1000 ml solution mix:

di-Sodium hydrogen phosphate dihydrate	17.80 g
Distilled water	1000 ml
dissolve	

Phosphate buffer solution pH 6

Mix 8.5 parts of stock solution A and 1.5 parts of stock solution B and filter.

Acridine orange solution

For preparation of approx. 100 ml solution mix:

Acridine orange zinc chloride double salt (C.I.46005) Certistain®	0.1 g
Distilled water	100 ml
dissolve and filter	

The freshly prepared staining solution should be filtered before use.

Acridine orange working solution

For preparation of approx. 100 ml solution mix:

Acridine orange solution	10 ml
Distilled water	90 ml
mix	

Acetic acid solution 1 %

For preparation of approx. 100 ml solution mix:

Acetic acid 100%	1 ml
Distilled water	99 ml
mix	

Calcium chloride differentiating solution 11 %

For preparation of approx. 100 ml solution mix:

Calcium chloride	1.1 g
Distilled water	100 ml
dissolve	

Procedure - Fluorochroming

Staining in the staining cell

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 fixed smear	
Ethanol 100 %	1 min
Ethanol 95 %	1 min
Ethanol 80 %	1 min
Ethanol 70 %	1 min
Distilled water	1 min
Acetic acid solution 1 %	rinse briefly
Distilled water	rinse
Phosphate buffer solution pH 6	2 min
Acridine orange working solution	3 min*
Allow excess dye to run off	
Phosphate buffer solution pH 6	2 min
Phosphate buffer solution pH 6	2 min
Mount with phosphate buffer, surround the edges with a cover glass, seal with colorless nail polish, view immediately under the microscope	

* Differentiate with calcium chloride solution 11 % to improve the fluorescence intensity of the cell nuclei if required.

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

Result

DNA-containing structures (nuclei, chromosomes, viral inclusions)	green to yellow-green fluorescence
RNA-containing structures (ribosomes, nucleoli, viral inclusions, acid mucous, mast cell granules)	red fluorescence

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 solution 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 Acridine orange zinc chloride double salt (C.I.46005) - for microscopy Certistain® at +5 °C to +30 °C.

Shelf-life

Acridine orange zinc chloride double salt (C.I.46005) - 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. 100921	Diethyl ether for analyses EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l, 4 l, 5 l
Cat. No. 100974	Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 l, 2.5 l
Cat. No. 102378	Calcium chloride anhydrous powder Reag. Ph Eur	500 g, 2.5 kg
Cat. No. 103699	Immersion oil acc. to ISO 8036 for microscopy	100-ml dropping bottle
Cat. No. 104699	Immersion oil for microscopy	100-ml dropping bottle, 100 ml, 500 ml
Cat. No. 104873	Potassium dihydrogen phosphate for analysis EMSURE® ISO	250 g, 1 kg
Cat. No. 106580	di-Sodium hydrogen phosphate dihydrate for analysis EMSURE®	500 g, 1 kg

Hazard classification

Cat. No. 1.15931.0025
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. 1.15931.0025
C.I. 46005
 $C_{17}H_{20}N_3 \times \frac{1}{2} ZnCl_4$
M = 438.11 g/mol

Other IVD products

Cat. No. 101374	Brilliant green (hydrogen sulfate) (C.I. 42040) for microscopy Certistain®	25 g
Cat. No. 103981	M-FIX® spray fixative for cytodiagnosis	100 ml, 1 l
Cat. No. 111674	Hemacolor® Rapid staining of blood smear staining set for microscopy	1 set

Literature

- Routine Cytological Staining Techniques: Theoretical Background and Practice, Mathilde E. Boon, Johanna S. Drijver, 1986, Elsevier Science Publishing Company
- 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: 2019-08-13

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