# Sigma-Aldrich<sub>®</sub>

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# **Microscopy**

# **Methyl blue (C.I. 42780)**

for microscopy



In Vitro Diagnostic Medical Device

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This staining dye "Methyl blue (C.I. 42780) - for microscopy" 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 evaluable for diagnostic purposes (by fixing, embedding where necessary, staining with the above methylblue solution, counterstaining, mounting).

#### **Principle**

Methyl blue belongs to the group of triaminotriphenylmethane dyes.

Methyl blue, aniline blue and water blue are usually mixtures of much the same components.

Methyl blue and aniline blue are so similar that both dyes are recorded under

C.I. 42780 and Color Index Name "Acid blue 93".

Water blue is recorded under C.I. 42755 with the Color Index Name "Acid blue 22".

Methyl blue, aniline blue and water blue are widely used in polychrome staining methods. The polychrome stains using especially mixtures with orange G or eosin. Besides methyl blue and aniline blues are used for staining of histones, for the determination of nuclear maturity, for demonstration of viability of sperms, resin embedded specimen can be stained with methyl blue also.

### Sample material

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

### Reagents

Cat. No. 116316

Methyl blue (C.I. 42780) 50 g, 1 kg for microscopy

Color Index No.: 42780 Color Index Name: Acid blue 93

### Also required:

Cat. No.	100583	Tungstophosphoric acid hydrate for analysis EMSURE®	100 g, 250 g
Cat. No.	100974	Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1  , 2.5
Cat. No.	105231	Fuchsin acid (C.I. 42685) for microscopy Certistain®	25 g
Cat. No.	115925	Orange G (C.I. 16230) for microscopy Certistain®	25 g

# Sample prepration

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

### **Fuchsin acid solution**

For preparation of approx. 100 ml solution mix:

Fuchsin acid (C.I. 42685) Certistain®	0.5 g
Distilled water	100 ml
dissolve and filter	

#### Methyl blue-Orange G solution

For preparation of approx. 100 ml solution mix:

Methyl blue (C.I. 42780)	0.15 g
Orange G (C.I. 16230) Certistain®	2 g
Tungstophosphoric acid hydrate	1 g
Distilled water	100 ml
dissolve and filter	

The freshly prepared staining solutions should be filtered before use.

#### **Procedure**

#### Polychrome staining

## 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 solu-

The stated times should be adhered to to guarantee an optimal staining

Slide with paraffin section		
Distilled water	1 min	
Fuchsin acid solution	5 min	
Methyl blue-Orange G solution	20 min	
Distilled water	rinse briefly	
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 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	red
Cytoplasm	pink
Collagen, mucin	blue

## **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 blue (C.I. 42780) - for microscopy at +5 °C to +30 °C.

# Shelf-life

Methyl blue (C.I. 42780) - for microscopy 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.

# **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

# Auxiliary reagents

Auxilia	ry reag	ents	
Cat. No.	100583	Tungstophosphoric acid hydrate for analysis EMSURE®	100 g, 250 g
Cat. No.	100974	Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 l, 2.5 l
Cat. No.	103699	Immersion oil acc. to ISO 8036 for microscopy	100-ml drop- ping bottle
Cat. No.	104699	Immersion oil for microscopy	100-ml drop- ping bottle, 100 ml, 500 ml
Cat. No.	105231	Fuchsin acid (C.I. 42685) 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
Cat. No.	109016	Neo-Mount® anhydrous mounting medium for microscopy	100-ml drop- ping bottle, 500 ml
Cat. No.	109843	Neo-Clear® (xylene substitute) for microscopy	5 I
Cat. No.	115925	Orange G (C.I. 16230) for microscopy Certistain®	25 g

# **Hazard classification**

Cat. No. 116316

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. 116316 C.I. 42780  $C_{37}H_{27}N_3Na_2O_9S_3$ M = 799.81 g/mol

# Other IVD products

<b>O</b> t	- P. O		
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.	105174	Hematoxylin solution modified acc. to Gill III for microscopy	500 ml, 1 l, 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.	115974	Elastica van Gieson staining kit for connective tissue	4x 500 ml
Cat. No.	117081	Eosin Y solution 1%, alcoholic for microscopy	1

### 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







Manufacturer



Catalog number







Temperature limitation

Status: 2019-09-20

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