

1.24823.0500

Microscopy

Nitro blue tetrazolium chloride (NBT)

for microscopy



In Vitro Diagnostic Medical Device



for staining of histological sections

This staining dye "Nitro blue tetrazolium chloride (NBT) - for microscopy" is used for human-medical cell diagnosis and serves 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 evaluable for diagnostic purposes (by fixing, embedding, staining with the above nitro blue solution, counterstaining, mounting) in histological specimen materials.

Principle

Nitro blue tetrazolium chloride belongs to the tetrazol dyes.

These dyes are used in histology, especially for histochemical methods, in vivo methods are additionally described.

Sample material

Sections of paraffin embedded tissue (3 - 5 µm thick paraffin sections) or cryo sections (for succinate dehydrogenase) are used as starting material.

Reagents

Cat. No. 1.24823.0500

Nitro blue tetrazolium chloride (NBT) 500 mg
for microscopy

Color Index No.: -

Color Index Name: -

Also required:

for vessel staining

Cat. No. 104201	Glycine GR for analysis	100 g, 250 g, 1 kg, 5 kg
Cat. No. 106404	Sodium chloride for analysis EMSURE® ACS,ISO,Reag. Ph Eur	500 g, 1 kg, 5 kg
Cat. No. 109959	Sodium hydroxide solution for 1000 ml, c(NaOH) = 0.1 mol/l (0.1 N) Titrisol®	1 amp

for succinate dehydrogenase staining

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. 103053	N,N-Dimethylformamide for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l, 4 l, 10 l
Cat. No. 104873	Potassium dihydrogen phosphate for analysis EMSURE® ISO	250 g, 1 kg
Cat. No. 105833	Magnesium chloride hexahydrate for analysis EMSURE® ACS,ISO,Reag. Ph Eur	250 g, 1 kg, 5 kg
Cat. No. 106437	Sodium cyanide EMPLURA®	1 kg
Cat. No. 106580	di-Sodium hydrogen phosphate dihydrate for analysis EMSURE®	500 g, 1 kg
Cat. No. 820151	di-Sodium succinate hexahydrate for synthesis	100 g, 500 g
Cat. No. M5625	Menadione crystalline	Sigma

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 paraffin sections in the conventional manner.

Vessel staining

Reagent preparation

Glycol buffer solution acc. to Sørensen pH 12.48

For preparation of approx. 200 ml solution mix:

Glycine	3.75 g
Sodium chloride	2.92 g
Distilled water	500 ml
dissolve	

Solution from above	75 ml
Sodium hydroxide solution 0.1 N	124 ml
mix	

Staining solution

For preparation of approx. 80 ml solution mix:

Nitro blue tetrazolium chloride (NBT)	20 mg
Glycol buffer solution acc. to Sørensen pH 12.48	80 ml
dissolve heat to 70 °C hot filter	

The freshly prepared staining solution should be filtered before use.

Procedure

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 solutions.

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

Slide with paraffin section	
Distilled water	1 min
Staining solution (56 - 60 °C)	60 - 90 min
Running tap water	5 min
Mount with Kaiser's glycerol gelatine and cover glass.	

Histological samples can be mounted with aqueous mounting agents (e.g. Kaiser's glycerol gelatine) 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

Vessel content, walls of the vessels (partly)	intense blue
Liver cells, heart cells (single cells)	red-violet
Erythrocytes	brown-red

Succinate dehydrogenase staining

Reagent preparation

Succinate solution

For preparation of approx. 1 ml solution mix:

di-Sodium succinate hexahydrate	270 mg
Distilled water	1 ml
dissolve pH should be 7.2 - 7.4. Store at -20 °C.	

Phosphate buffer solution pH 7.2

Stock solution A - potassium dihydrogen phosphate buffer 0.1 M

For preparation of approx. 1000 ml solution mix:

Potassium dihydrogen phosphate (KH ₂ PO ₄)	13.61 g
Distilled water	1000 ml
dissolve	

Stock solution B - di-Sodium hydrogen phosphate dihydrate buffer 0.1 M

For preparation of approx. 1000 ml solution mix:

di-Sodium hydrogen phosphate dihydrate (Na ₂ HPO ₄ x 2 H ₂ O)	17.8 g
Distilled water	1000 ml
dissolve	

Phosphate buffer solution pH 7,2

For preparation mix:

Stock solution A	3 parts
Stock solution B	7 parts
mix	

Nitro blue tetrazolium chloride staining solution

For preparation of approx. 20 ml solution mix:

Nitro blue tetrazolium chloride (NBT)	20 - 80 mg
N,N-Dimethylformamide	1 ml
dissolve	
Distilled water	19 ml
add, mix and filter, if necessary	

Sodium cyanide solution

For preparation of approx. 100 ml solution mix:

Sodium cyanide	0.05 g
Distilled water	100 ml
dissolve	
Measure the pH and bring to pH 7.2 with HCl, if necessary.	

Magnesium chloride solution

For preparation of approx. 100 ml solution mix:

Magnesium chloride hexahydrate	1 g
Distilled water	100 ml
add and mix	

Nitro blue tetrazolium chloride stock solution

For preparation of approx. 18 ml solution mix:

Phosphate buffer solution pH 7,2	10 ml
Nitro blue tetrazolium chloride staining solution	10 ml
Sodium cyanide solution	4 ml
Magnesium chloride solution	4 ml
mix, filter, if necessary, and store in the refrigerator	

Menadione solution

For preparation of approx. 100 ml solution mix:

Menadione crystalline	0.5 g
Distilled water	100 ml
dissolve	

Incubation solution

For preparation of approx. 4 ml solution mix:

Nitro blue tetrazolium chloride stock solution	4 ml
Succinate solution	0.2 - 0.4 ml
Menadione solution	4 - 6 drops
mix	

The freshly prepared staining solutions should be filtered before use, if necessary.

Procedure

Staining on the staining rack

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

Slide with cryo section			
Incubation solution	2 ml	apply incubate at room temperature	5 - 45 min
Distilled water		rinse	
Formaldehyde solution 4%		fix	
Running tap water		rinse	
Mount with Karion® and cover glass.			

Histological samples can be mounted with Karion® 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

Succinate dehydrogenase blue

Technical notes

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

When using 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 Nitro blue tetrazolium chloride (NBT) - for microscopy at +2 °C to +8 °C.

Shelf-life

Nitro blue tetrazolium chloride (NBT) - 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 +2 °C to +8 °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.

If necessary use a standard centrifuge suitable for medical diagnostic laboratory.

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. 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. 102993	Sorbitol F liquid (sorbitol syrup, noncrystallizing) E 420 (Karion®)	50 kg
Cat. No. 103053	N,N-Dimethylformamide for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l, 4 l, 10 l
Cat. No. 103693	Cryoembedding media for microscopy M-FREEZE™	100 ml
Cat. No. 103699	Immersion oil acc. to ISO 8036 for microscopy	100-ml dropping bottle
Cat. No. 104201	Glycine GR for analysis	100 g, 250 g, 1 kg, 5 kg
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. 105833	Magnesium chloride hexahydrate for analysis EMSURE® ACS,ISO,Reag. Ph Eur	250 g, 1 kg, 5 kg
Cat. No. 106404	Sodium chloride for analysis EMSURE® ACS,ISO,Reag. Ph Eur	500 g, 1 kg, 5 kg
Cat. No. 106437	Sodium cyanide EMPLURA®	1 kg
Cat. No. 106580	di-Sodium hydrogen phosphate dihydrate for analysis EMSURE®	500 g, 1 kg
Cat. No. 108103	Tetrazolium blue for microscopy	5 g
Cat. No. 108635	Kaiser's glycerol gelatine, phenol-free for microscopy	100-g dropping bottle
Cat. No. 109242	Kaiser's glycerol gelatine for microscopy	100 g
Cat. No. 109959	Sodium hydroxide solution for 1000 ml, c(NaOH) = 0.1 mol/l (0.1 N) Titrisol®	1 amp
Cat. No. 820151	di-Sodium succinate hexahydrate for sythesis	100 g, 500 g
Cat. No. M5625	Menadione crystalline	Sigma

Safety classification

Cat. No. 1.24823.0500

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 products

Cat. No. 1.24823.0500

C.I. -

$C_{40}H_{30}Cl_2N_{10}O_6$

M = 817.7 g/mol

Other IVD products

Cat. No. 102419	Oil red O color solution for the detection of neutral lipids in cryo sections for microscopy	250 ml
Cat. No. 103999	Formaldehyde solution min. 37% free from acid stabilized with about 10% methanol and calcium carbonate for histology	1 l, 2.5 l, 5 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: 2019-09-20

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

