

1.11732.0025

Microscopy

Trypan blue (C.I. 23850)

for microscopy

IVD

In Vitro Diagnostic Medical Device

CE

This staining dye “Trypan blue (C.I. 23850) - 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 (by fixing, embedding, staining with the above trypan blue solution, counterstaining, mounting) in histological specimen materials evaluable for diagnostic purposes.

Principle

Trypan blue is a large anionic dye that belongs to the group of azo dyes. Trypan blue is traditionally used as a component of polychromatic stains, e.g. for demonstration of collagenous connective tissue. Trypan blue is described in the literature as a dye for vital stains.

Sample material

Sections of formalin-fixed, paraffin-embedded tissue (3 - 4 µm thick paraffin sections) are used as starting material.

Reagents

Cat. No. 1.11732.0025	
Trypan blue (C.I. 23850) for microscopy	25 g
Color Index No.:	23850
Color Index Name:	Direct blue 14, Niagara blue 3B

Also required:

Cat. No. 100317	Hydrochloric acid fuming 37% for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l, 25 l
Cat. No. 105231	Fuchsin acid (C.I. 42685) for microscopy Certistain®	25 g
Cat. No. 115973	Weigert's iron hematoxylin kit for nuclear staining in histology	2x 500 ml
Cat. No. 70540	Naphthol Yellow S	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 sections in the conventional manner.

Reagent preparation

Naphthol Yellow S solution

For preparation of approx. 100 ml solution mix:

Naphthol Yellow S	0.3 g
Distilled water	100 ml
dissolve and filter	

Fuchsin acid solution

For preparation of approx. 100 ml solution mix:

Fuchsin acid (C.I. 42685) Certistain®	0.2 g
Distilled water	100 ml
dissolve and filter	

Trypan blue solution

For preparation of approx. 100 ml solution mix:

Trypan blue (C.I. 23850)	0.2 g
Distilled water	100 ml
dissolve and filter	

Staining solution

For preparation of approx. 90 ml solution mix:

Naphthol Yellow S solution	80 ml
Fuchsin acid solution	5 ml
Trypan blue solution	4 ml
Hydrochloric acid fuming 37%	0.8 ml
mix and filter	

The freshly prepared staining solutions should be filtered before use.

Weigert's iron hematoxylin staining solution

Mix reagent 1 and 2 (Weigert's solution A and Weigert's solution B) in the ratio 1 + 1.

The prepared staining solution remains stable for approx. one working week. The solution must be exchanged as soon as the cell nuclei appear brown.

Procedure

Trichrome 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
Weigert's iron hematoxylin staining solution	5 min
Running tap water	5 min
Staining solution	10 min
Distilled water	rinse
Ethanol 50 %	1 min
Ethanol 70 %	1 min
Ethanol 96 %	1 min
Ethanol 100 %	1 min
Ethanol 100 %	1 min
Xylene	1 min
Xylene	1 min
Mount the xylene-wet slides with e.g. Entellan® new and cover glass.	

After dehydration (ascending alcohol series) and clarification with xylene, histological slides can be covered with non-aqueous mounting agents (e.g. Entellan® new) 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

Nuclei	blue-black
Cytoplasm	yellow
Collagenous connective tissue	
large fibers	red
small fibers	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 Trypan blue (C.I. 23850) - for microscopy at +5°C to +30°C.

Shelf-life

Trypan blue (C.I. 23850) - 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 °C to +30 °C.

The bottles must be kept tightly closed at all times.

If stored at +15 °C to +25 °C, the freshly prepared Weigert's iron hematoxylin staining solution can be used for minimum one working week.

The solution must be exchanged as soon as the cell nuclei appear brown.

However, the solutions should be discarded when contaminations (e.g. fungi, bacteria), that occur at times, are observed.

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.	100317	Hydrochloric acid fuming 37% for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l, 25 l
Cat. No.	104699	Immersion oil for microscopy	100-ml dropping bottle, 100ml, 500 ml
Cat. No.	105231	Fuchsin acid (C.I. 42685) for microscopy Certistain®	25 g
Cat. No.	107960	Entellan® rapid mounting medium for microscopy	500 ml
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.	115973	Weigert's iron hematoxylin kit for nuclear staining in histology	2x 500 ml
Cat. No.	70540	Naphthol Yellow S	Sigma

Safety classification

Cat. No. 1.11732.0025

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 products

Cat. No. 1.11732.0025

C.I. 23850

$C_{34}H_{24}N_6Na_4O_{14}S_4$

M = 960.80 g/mol

Other IVD products

Cat. No.	100199	Picrofuchsin solution acc. to van Gieson for microscopy	500 ml
Cat. No.	100485	Masson-Goldner staining kit for the visualization of connective tissue with trichromic staining	1 unit
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.	115161	Histosec® pastilles (without DMSO) solidification point 56-58°C embedding agent for histology	10 kg (4x 2.5 kg), 25 kg
Cat. No.	115974	Elastica van Gieson staining kit for connective tissue	4x 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. 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: 2017-08-15

Merck KGaA, 64271 Darmstadt, Germany
Tel. +49(0)6151 72-2440
www.microscopy-products.com

EMD Millipore Corporation, 290 Concord Road, Billerica,
MA 01821, USA, Tel. +1-978-715-4321

