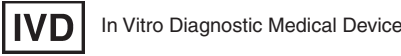


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Microscopy

Giemsa’s azur eosin methylene blue solution

for microscopy



for the staining of blood and bone-marrow smears, paraffin sections and clinical-cytological specimens

This “Giemsa’s azur eosin methylene blue solution - for microscopy” is used for human-medical cell diagnosis and serves the investigation of sample material of human origin. It is a staining solution that when used together with other in vitro diagnostic products from our portfolio makes target structures (by fixing, embedding, staining, counterstaining, mounting) in human-hematological, histological and clinico-cytological specimen materials, for example smears of whole blood and bone marrow, as well as paraffin sections, evaluable for diagnostic purposes.

Principle

Giemsa’s stain is frequently used for diagnostic purposes in the areas of hematology and histology.

When used in hematological applications, Giemsa’s stain is frequently used in combination with other dye solutions, e.g. with May-Grünwald’s solution for Pappenheim (MGG) overview staining. This staining solution generally stains the nuclei red, based on the molecular interaction between the eosin Y dye and an Azure B-DNA complex. Both dyes assemble to an Eosin Y - Azure B-DNA complex and the intensity of the resulting stain depends on the content of Azure B and the ratio of Azure B : Eosin Y.

Furthermore, the resulting stain can vary depending on the influence of fixation, staining times, pH-value of the solutions or buffer substances.

In histology and clinico-cytological applications, Giemsa’s staining without additional dyes is used as an extended overview staining method. In this method, the color of the various cell components is influenced by pretreatment of the specimen material. Here, cell nuclei appear in various blue shades, while the acidophilic components show up in a variety of red shades.

Sample material

Sections of formalin fixed, paraffin embedded tissue (3 - 4 µm thick paraffin sections) or fresh, native whole blood and bone-marrow smears just as clinical cytological material like urine sediment, sputum, smears from fine needle aspiration biopsies (FNAB), rinses, imprints are used as starting material.

Reagents

Art. 109204
Giemsa’s azur eosin methylene blue solution 100 ml, 500 ml, 1 l, 2,5 l
for microscopy

Also required:

Cat. No.	106009	Methanol for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2,5 l, 5 l
Cat. No.	109468	Buffer tablets pH 7.2 for preparing buffer solution acc. to WEISE for staining of blood smears	100 tabs
or			
Cat. No.	111373	Buffer tablets pH 6.4 for preparing buffer solution acc. to WEISE for the staining of blood smears	100 tabs
or			
Cat. No.	111374	Buffer tablets pH 6.8 for preparing buffer solution acc. to WEISE for the staining of blood smears	100 tabs

for the staining of paraffin sections:			
Cat. No.	100063	Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2,5 l
Cat. No.	109634	2-Propanol for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2,5 l, 5 l
for the Pappenheim staining:			
Cat. No.	101424	May-Grünwald’s eosine-methylene blue solution modified for microscopy	100 ml, 500 ml, 1 l, 2,5 l

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.

Notes on Giemsa staining of paraffin sections

Always employ separate xylene or Neo-Clear® (Cat. No. 109843) rinse baths when Giemsa staining paraffin sections as any ethanol traces in the solutions may result in the preparations being discolored.

Pretreatment of bone marrow and iliac crest biopsy materials

Optimal results can be achieved using a OSTEOSOFT® mild decalcifier-solution (Cat. No. 101728).

To gently remove any calcification, the fixed biopsy materials are first placed in OSTEOSOFT® for 18 - 24 hours, after which they are transferred to histoprocessing. Blocks are carefully cut and, if required, are again treated with OSTEOSOFT® for an additional 20 minutes.

Reagent preparation

Giemsa’s azur eosin methylene blue solution

The solution is supplied as a concentrated staining solution and before use must be diluted with a buffer solution as described below. The diluted color solution should be filtered before use.

When performing the **Giemsa rapid staining procedure** (for clinical and inter-operative smears) and **Giemsa staining** (for paraffin sections of punched iliac crest specimens and the detection of Helicobacter pylori) the solution is used undiluted. It must, however, be filtered before use.

Buffer solution

For preparation of approx. 1000 ml of solution, add and dissolve:

Buffer tablet, Cat. No. 111373 (pH 6.4), Cat. No. 111374 (pH 6.8) or Cat. No. 109468 (pH 7.2) depending on the required reaction color	1 tablet
Distilled water	1000 ml

Dilute Giemsa’s staining solution for manual staining in the staining cell

For preparation of approx. 200 ml solution mix:

Giemsa’s azur eosin methylene blue solution	10 ml
Buffer solution	190 ml
Mix well, leave to stand for 10 min, and filter if necessary	

Dilute Giemsa’s staining solution for staining with staining automate

For preparation of approx. 300 ml solution mix:

Giemsa’s azur eosin methylene blue solution	25 ml
Buffer solution	275 ml
Mix well, leave to stand for 10 min, and filter if necessary	

In many cases precipitates of the dye form in the diluted staining solutions; these can be eliminated by repeating the filtration process.

Acetic acid 0.1 %, aqueous

For preparation of approx. 1000 ml solution mix:

Acetic acid 100 %	1 ml
Distilled water	1000 ml

Giemsa staining

Procedure

Air-dried smears

Staining in the staining cell / on the staining rack

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 air-dried smear	
Methanol	3 min
Dilute Giemsa’s staining solution for manual staining	20 min
Buffer solution	1 min
Buffer solution	1 min
Air-dry (e.g. over night or at 50°C in the drying cabinet)	

Staining in the staining automate

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

	Time	Station	Dip
Slide with air-dried smear			
Methanol	3 min	2	on
Dilute Giemsa’s staining solution for staining with staining automate	20 min	3	on
Buffer solution	1 min	4	on
Running tap water	2 min	5	on
Dry	3 min	6	-

Covering with non-aqueous mounting media (e.g. Neo-Mount®, DPX new, or Entellan® new) and a cover glass is recommended for the storage of hematological specimens for several months. When left unmounted, the stain remains stable for approx. 3 days, covered with immersion oil for just a few hours.

After dehydration (ascending alcohol series) and clarification with xylene or Neo-Clear®, cytological samples can be mounted with water-free mounting agents (e.g. Entellan® new, DPX 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

	Buffer solution pH 6.4	Buffer solution pH 6.8	Buffer solution pH 7.2
Cell nuclei	red to violet	red to violet	violet
Lymphocytes	plasma blue	plasma blue	plasma blue
Monocytes	plasma grey-blue	plasma grey-blue	plasma grey-blue
neutrophilic granulocytes	granule light violet	granule light violet	granule light violet
eosinophilic granulocytes	granule reddish to red-brown	granule reddish to red-brown	granule reddish to red-brown
basophilic granulocytes	granule dark violet	granule dark violet	granule dark violet
Thrombocytes	violet	violet	violet
Erythrocytes	reddish	reddish	reddish-brownish
Blood parasites	cell nuclei light red	cell nuclei light red	cell nuclei light red

Pappenheim staining

with May-Grünwald’s solution and Giemsa’s solution

Procedure

Air-dried smears

Staining in the staining cell

The slides must be immersed and moved briefly in the solutions, simple immersion alone yields inadequate staining results.

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 air-dried smear	
May-Grünwald’s eosine-methylene blue solution modified	3 min
Dilute Giemsa’s staining solution for manual staining	20 min
Buffer solution	1 min
Buffer solution	1 min
Air-dry (e.g. over night or at 50°C in the drying cabinet)	

Staining on the staining rack

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

Slide with air-dried smear			
May-Grünwald’s eosine-methylene blue solution modified		cover completely	3 min
Buffer solution	1 ml	mix	
Dilute Giemsa’s staining solution for manual staining		cover completely	20 min
Buffer solution		rinse	
Air-dry (e.g. over night or at 50°C in the drying cabinet)			

Covering with non-aqueous mounting media (e.g. Neo-Mount®, DPX new, or Entellan® new) and a cover glass is recommended for the storage of hematological specimens for several months. When left unmounted, the stain remains stable for approx. 3 days, covered with immersion oil for just a few hours.

After dehydration (ascending alcohol series) and clarification with xylene or Neo-Clear®, cytological samples can be mounted with water-free mounting agents (e.g. Entellan® new, DPX 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

	Buffer solution pH 6.8	Buffer solution pH 7.2
Cell nuclei	purple to violet	violet
Lymphocytes	plasma blue	plasma blue
Monocytes	plasma grey-blue	plasma grey-blue
neutrophilic granulocytes	granule light violet	granule violet
eosinophilic granulocytes	granule brick-red	granule red-brown
basophilic granulocytes	granule dark violet to black	granule dark violet to black
Thrombocytes	violet	violet
Erythrocytes	reddish	reddish-grey

Giemsa rapid staining

Procedure

Clinical and interoperative smears

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 air-dried smear	
Methanol	1 min
Giemsa's azur eosin methylene blue solution (undiluted, filtered)	1 min
Buffer solution pH 6,8	1 min
Buffer solution pH 6,8	1 min
Remove the specimen from the buffer solution and mount moist with a cover glass, and microscope immediately.	

For storage, it is advisable to mount the specimen with a mounting medium and a cover glass. To do this remove the cover glass after microscoping, rinse the specimen briefly in buffer solution, and after drying mount with an anhydrous mounting medium (e.g. Neo-Mount®, DPX Neu, Entellan® New) and a cover glass.

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

Result

Chromatin	pink to blue
Cytoplasm	blue (in various shades)
Connective tissue	tender red
acidophilic granula	light red
Hemoglobin	bright red

Giemsa staining

Procedure

Paraffin sections of punched iliac crest specimens and detection of Helicobacter pylori

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.

Deparaffinize histological slides in the conventional manner and rehydrate in a descending alcohol series.

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

Always employ separate xylene or Neo-Clear® (Cat. No. 109843) rinse baths when Giemsa staining paraffin sections as any ethanol traces in the solutions may result in the preparations being discolored.

Slide with histological specimen	
Distilled water	10 sec
Giemsa's azur eosin methylene blue solution (undiluted, filtered)	15 min
Acetic acid 0.1 %	10 sec
Distilled water	10 sec
2-Propanol	10 sec
2-Propanol	10 sec
2-Propanol	10 sec
Xylene or Neo-Clear®	5 min
Xylene or Neo-Clear®	5 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, 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

Cell nuclei, cells	blue to dark blue
Collagen, osteoid	pale blue
Eosinophilic granula	red
Acidic mucopolysaccharides, mastocytes granula, cartilage matrix	reddish-violet
Acidophilic materials	orange-red
Helicobacter pylori	blue to dark 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 diluted 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 the Giemsa's azur eosin methylene blue solution - for microscopy at +15 °C to +25 °C.

Shelf-life

The Giemsa's azur eosin methylene blue solution - for microscopy the stated expiry date.

After first opening of the bottle, the contents can be used up to the stated expiry date when stored at +15 °C to +25 °C.

The bottles must be kept tightly closed at all times.

Capacity

3500 - 5000 stainings / 500 ml

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.	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.	100579	DPX new non-aqueous mounting medium for microscopy	500 ml
Cat. No.	100974	Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 l, 2.5 l
Cat. No.	101424	May-Grünwald's eosine-methylene blue solution modified for microscopy	100 ml, 500 ml, 1 l, 2.5 l
Cat. No.	101728	OSTEOSOFT® mild decalcifier-solution for histology	1 l, 10 l Titripac®
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.	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.	109203	Giemsa's azur-eosin- methylene blue for microscopy	25 g, 100 g
Cat. No.	109468	Buffer tablets pH 7.2 for preparing buffer solution acc. to WEISE for staining of blood smears	100 tabs
Cat. No.	109634	2-Propanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 l, 2.5 l, 5 l
Cat. No.	109843	Neo-Clear® (xylene substitute) for microscopy	5 l
Cat. No.	111373	Buffer tablets pH 6.4 for preparing buffer solution acc. to WEISE for the staining of blood smears	100 tabs
Cat. No.	111374	Buffer tablets pH 6.8 for preparing buffer solution acc. to WEISE for the staining of blood smears	100 tabs
Cat. No.	111609	Histosec® pastilles solidification point 56-58°C embedding agent for histology	1 kg, 10 kg (4x 2.5 kg), 25 kg
Cat. No.	115161	Histosec® pastilles (without DMSO) solidification point 56-58°C embedding agent for histology	10 kg (4x 2.5 kg), 25 kg

Hazard classification

Cat. No. 109204

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. 109204

C.I.52015 + Azure 4.1 g/l
C.I.45380 2.4 g/l

contains CH₃OH

1 l = 0.99 kg

Other IVD products

Cat. No.	100869	Entellan® new for cover slipper for microscopy	500 ml
Cat. No.	101383	Wright's eosin methylene blue solution for microscopy	100 ml, 500 ml, 2.5 l
Cat. No.	102439	Eosin Y-solution 0.5%, alcoholic for microscopy	500 ml, 2.5 l
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, 25 l
Cat. No.	105174	Hematoxylin solution modified acc. to Gill III for microscopy	500 ml, 1 l, 2.5 l
Cat. No.	105175	Hematoxylin solution modified acc. to Gill II for microscopy	500 ml, 2.5 l
Cat. No.	105387	Leishman's eosin methylene blue solution modified for microscopy	500 ml
Cat. No.	109844	Eosin Y-solution 0.5% aqueous for microscopy	1 l, 2.5 l
Cat. No.	111661	Rapid staining of blood smear staining kit for microscopy	1 set
Cat. No.	117081	Eosin Y solution 1%, alcoholic for microscopy	1 l

Literature

1. Löffler, H., Rastetter, J., Haferlach, T, Atlas der klinischen Hämatologie, 2004, Springer-Verlag Berlin Heidelberg
2. Routine Cytological Staining Techniques: Theoretical Background and Practice, Mathilde E. Boon, Johanna S. Drijver, 1986, Elsevier Science Publishing Company
3. Romeis - Mikroskopische Technik, Editors: Mulisch, Maria, Welsch, Ulrich; 2015; Springer-Verlag Berlin Heidelberg
4. Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble, 6th Edition
5. 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: 2018-01-26

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