ENGLISH

DIAPATH

Special Stains Handbook

DIAPATH

PREFACE

Diapath S.p.A. is glad to introduce the "Special Stains Handbook".
In this handbook are collected the components and the main staining protocols of special stains kit.
Special stains are generally the non-routine stains applied to histological sections or cytological preparations, able to show specific tissue components, to differentiate cell types and detect the presence of any microorganisms.
Special stains are, therefore, a good tool to support the research techniques normally used to define the diagnostic patient profile.

This handbook provides practical advice for getting the most from different staining techniques and offers the best solutions to overcome the most common mistakes.

Before proceeding, we point out some important notes for a quick reading.
In each staining protocols (and in the corresponding data sheet) is used the wording "*Deparaffinize and hydrate to distilled water*" referring to the deparaffinizing steps (in xylene or substitutes) and hydration (ascending alcoholic scale with last step in water) of the section. In some cases the complete hydration is not necessary, but it will be mentioned, for example, the step in which start the staining."*Dehydrate, clear and mount with balsam*" are the further steps in the ascending alcoholic scale and xylene (or substitutes) that allow a steady slide mounting.
If it is necessary the use of a water-based mounting media, it will be specified in the staining protocol.
The suggested times are an approximation and could change according to specific needs.

The special stains kits are planned for about 100 tests, considering that to cover a specimen are necessary about 10 drops. Please note that the drop number changes according to specimen size.
On request, are available kits with larger packaging to perform staining in immersion.
Some solutions should be prepared only shortly before their use to avoid possible degrade that could affect staining result.

To avoid undesirable specimen drying, we recommend to perform staining keeping the coverslip in an incubation box.
We suggest the use of control tests to verify that the protocol is properly performed.

The special stain kits are supplied in special packaging with practical containers which allow an application drop by drop on the specimen, ensuring a considerable material saving.
The Technical Data Sheet with the staining protocol is attached to each package.

Here follows the necessary equipment to be used during the staining protocols (not supplied in the kit):

- Incubation box with rack for horizontal staining. Alternatively, we suggest the use of an humidified slide box on the bottom with some blotting paper soaked in water
- Squeeze bottle with distilled water or vertical Choplin jar filled with 50 ml of distilled water for the washing steps
- Reagents for deparaffinizing: xylene or substitutes
- Reagents for dehydration, hydration, clarification and mounting: alcoholic scale, xylene or substitutes, mounting media, coverslip
- Blotting paper
- Additional glassware: histological Choplin jar, glass stick, rectangular jars for staining. We recommend the use of clean glassware. In case of protocols involving the use of reagents containing silver, don't use metallic objects.

We hope that this handbook is a valuable guide for your histological procedures and a valuable help to perform special staining.

Good Reading

The image shades reported here can change according to the employed printing process.

RECEIVE IN REAL TIME ALL DIAPATH SPECIAL STAINS UPDATINGS

Diapath Special Stains Handbook is a constantly updating catalogue. Visit our website **www.diapath.com**, register in the Reserved Area and ask to be a member of our mailing list to receive in real time all Diapath special stains updatings.
Contact our Product Specialists at **specialstains@diapath.com** to receive support, information and explanations concerning Diapath special stains protocols and methods.

CLASSIFICATION ACCORDING TO TISSUE KIND

CONNECTIVE TISSUE	code
Azan Trichrome (renal biopsies)	010212
AFOG (Acid Fuchsine Orange G) stain (renal biopsies)	010307
Goldner trichrome (Masson's trichrome with light green)	010224
Silver impregnation	010211
Mallory's trichrome acc. Mc Farlane	010227
Masson's trichrome	010210
Movat pentachrome stain (collagen, mucins, reticular fibers)	010247
Acid Orcein (elastic fibers)	010251
P.A.S.M.- Silver Methenamine acc. Callard (basal membrane)	010234
Picro Mallory trichrome acc. Lendrum	010238
P.T.A.H. Phosphotungstic acid hematoxylin acc. Mallory	010239
Van Gieson Trichrome acc. Weigert	010240
Verhoeff's stain (elastic fibers)	010308
Wiegert for elastic fibers, fast method	010242
Wiegert for elastic fibers, long method	010217
Weigert-Van Gieson, long method (connective tissue and elastic fibers)	010218
Weigert-Van Gieson, fast method (connective tissue and elastic fibers)	010243
Paraldehyde fuchsin acc. Gomori (pancreas)	010235
Sirius Red for collagen	010254
Gomori's trichrome (muscle)	010302

CARBOHYDRATES	code
Alcian Blue pH 0.2 acc. Dorling (mucins)	010203
Alcian Blue pH 0.5 acc. Dorling (mucins)	010204
Alcian Blue pH 1.0 acc. Dorling (mucins)	010205
Alcian Blue pH 1.5 acc. Dorling (mucins)	010206
Alcian Blue pH 2.5 acc. Dorling (mucins)	010207
Alcian Blue pH 3.1 acc. Dorling (mucins)	010208
P.A.S. (Periodic Acid Schiff) acc. Hotchkiss-McManus (glycogen)	010231
P.A.S. (Periodic Acid Schiff) acc. Morel-Maronger (glycogen)	010232
P.A.S. (Periodic Acid Schiff) acc. Pearse (glycogen)	010233
Alcian blue pH 2.5 – P.A.S. acc. Mowry (mucins and glycogen)	010209
Mucicarmine acc. Mayer (mucins)	010245
Congo Red (amyloid)	010214
Sirius Red for amyloid	010306
Dane trichrome (mucins and keratin)	010215
Diastase Buffer (pre-treatment for P.A.S.)	010216

CENTRAL NERVOUS SYSTEM	code
Luxol fast blue acc. Kluwer-Barrera	010226

PIGMENTS AND MINERAL DEPOSITS	code
Grimelius (argyrophil cells)	010222
Fouchet-Van Gieson acc. Kutlick (bilirubin)	010220
Masson Fontana (melanin)	010228
Perls (ferric iron)	010236
Perls-Van Gieson (ferric iron and connective tissue)	010237
Hale reaction (colloidal iron)	010312
Rhodamine (copper)	010248
Von Kossa acc. McGee-Russel (calcium)	010241

FUNGI AND BACTERIA	code
Gram for histological sections (bacteria)	010221
Twort's stain (bacteria)	010310
Grocott acc. Callard (fungi)	010223
May Grunwald Giemsa acc. Romanowsky for tissue sections	010229
Acid Fast Bacteria acc. Ziehl-Neelsen modified acc. Fite (mycobacteria)	010202
Acid Fast Bacteria acc. Ziehl-Neelsen (mycobacteria)	010201
Alcian yellow-Toluidine Blue (*Helicobacter pylori*)	010269
Long Giemsa acc. Lennert (*Helicobacter pylori*)	010225
Warthin Starry	010270

NUCLEI AND NUCLEIC ACIDS	code
AgNOR	010801
Feulgen and Rossenbeck	010219

LIPIDS	code
Oil Red O acc. Johnson	010303

STAINING FOR CITOLOGY	code
May Grunwald Giemsa for smears	010802
Fast Quick - M.G.G. Rapid	010253

CRYOSTAT	code
Fast Quick Hematoxylin Eosin	010263
Oil Red O acc. Johnson (lipids)	010303
Gomori's trichrome (muscle)	010302

WARNING: The reported protocols may require incubation time changes according to laboratory needs.

HISTOLOGICAL STAINING KIT

ACID FAST BACTERIA acc. ZIEHL-NEELSEN

code 010201

IVD CE

Description

The Kit supplies reagents for the staining protocol according to Ziehl-Neelsen. This stain is particularly suitable to highlight acid-resistant bacteria such as mycobacteria, *Nocardia* and parasites in histological sections, smears, cultures and expectorations. The staining protocol is based on the characteristic structure of acid-resistant bacteria that acquires and retains red stain.
The Kit code 010201 is characterized by counterstaining with Mayer Hematoxylin.

Staining protocol

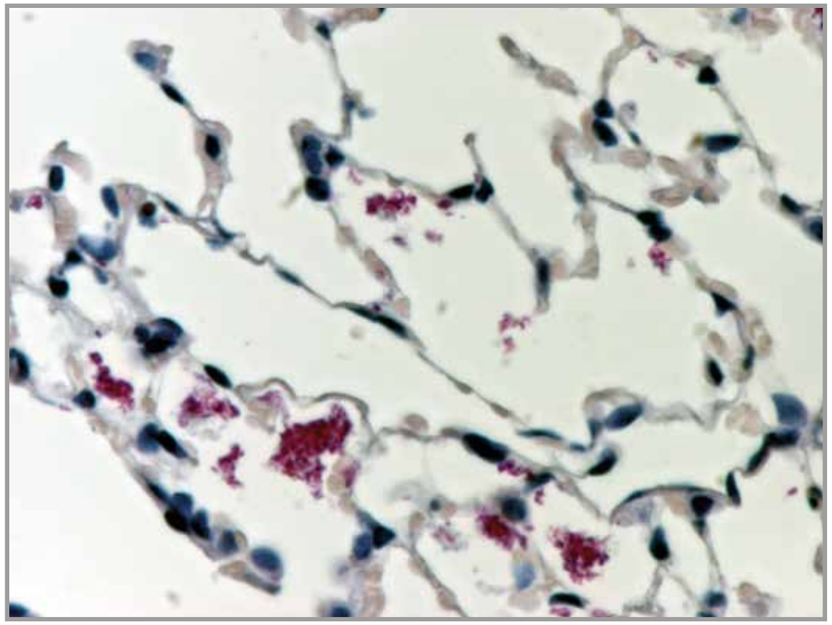
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. Dilute 15 drops of **reagent A (Carbolfuchsin)** in 1 ml of distilled water
3. Cover the section with the solution for 30 minutes at room temperature
4. Remove liquid in excess from the slide
5. Dilute 10 drops of **reagent B (Differentiation Solution)** in 95 ml of distilled water
6. Immerse slides in the solution for 1-2 minutes until the complete section discoloration
7. Wash in running tap water for 3 minutes
8. **Reagent C (Mayer Hematoxylin)** for 2 minutes
9. Tone in running tap water for at least 5 minutes
10. Dehydrate quickly, clear and mount with balsam

Results

Acid-resistant bacteria: Red
Nuclei: Blue-Violet

Preparation	Paraffin section
Control	Tissues with acid-alcohol resistant bacteria
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	45 min
Suggested fixatives	Formalin
Critical step	Avoid section drying



Mouse lung. Positive case for bacteria shown in red.

HISTOLOGICAL STAINING KIT

ACID FAST BACTERIA acc. ZIEHL-NEELSEN modified acc. FITE
code 010202

IVD CE

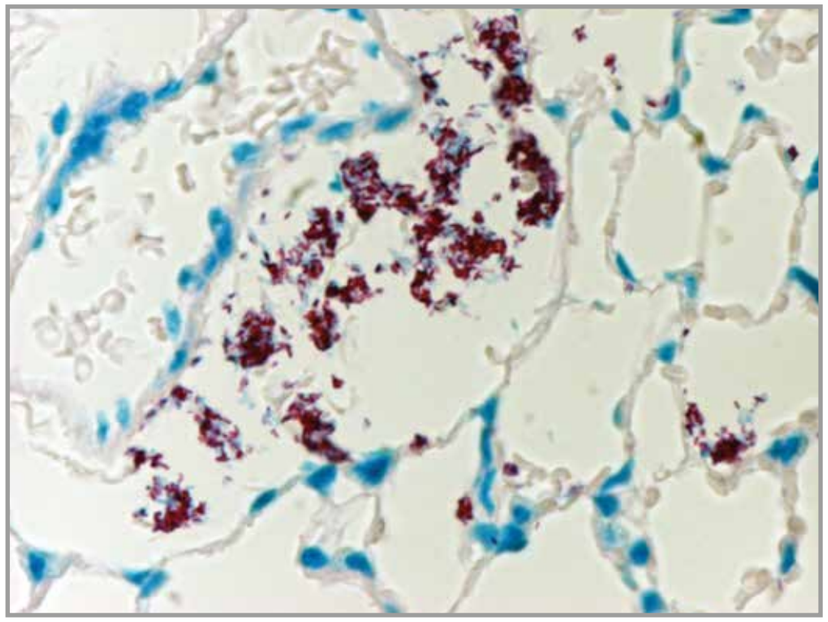
Description
The Kit supplies reagents for Ziehl-Neelsen modified according Fite staining protocol. This stain is particularly suitable to highlight acid-resistant bacteria such as mycobacteria, *Nocardia* and parasites in histological sections, smears, cultures, expectorations and *Mycobacterium Leprae* (Leprosy etiological agent). The staining protocol is based on the characteristic structure of acid-resistant bacteria that acquires and retains red stain.
The Kit code 010202 is characterized by counterstaining with methylene blue.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. **Reagent A (Periodic Acid)** for 15 minutes
3. Wash in distilled water
4. **Reagent B (Carbolfuchsin)** for 30 minutes
5. Wash in distilled water
6. **Reagent C (Differentiation Solution)** for 1 minute until the section doesn't loose pink stain
7. Wash in running tap water for 5 minutes
8. Prepare countersolution: 5 drops of **reagent D (Methylene blue)** + 5 drops of **reagent E (Basic Buffer)**
9. Cover the section with the solution for 30 seconds
10. Wash in running tap water for 1-2 minutes
11. Dehydrate quickly, clear and mount with balsam

Results
Acid-resistant bacteria: Red
Background: Blue

Preparation	Paraffin section
Control	Bactery infection
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	55 min
Suggested fixatives	Formalin
Critical step	Avoid section drying



Mouse lung. Positive case for bacteria shown in red.

HISTOLOGICAL STAINING KIT

ACID ORCEIN
code 010251

IVD CE

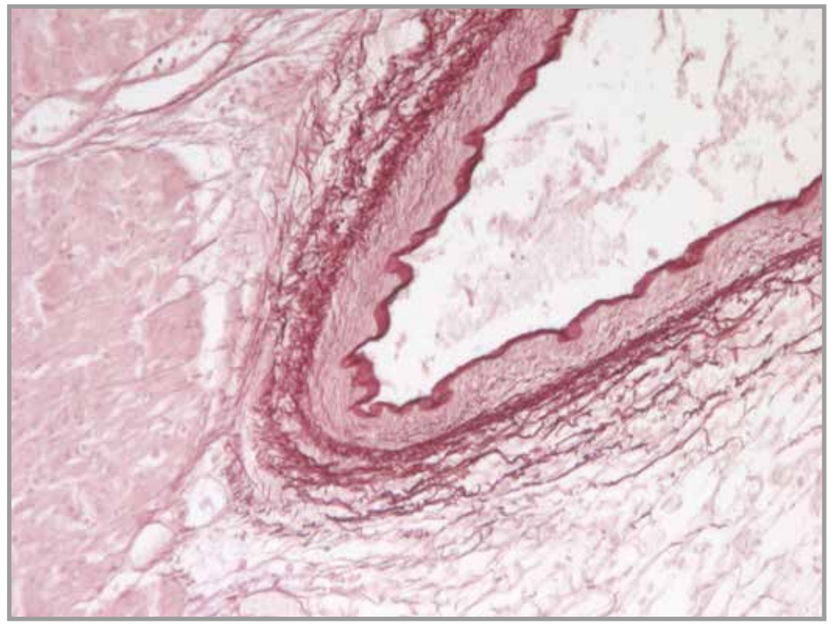
Description
The Kit is intended for use in histological visualization of elastic fibers with acid orcein. If used to visualize Australia Antigen (HBsAg, Hepatitis B Surface Antigen) specific of hepatitis B virus, the result must always be supported by immunohistochemical investigation. The elastic fibers are visualized by different special stains. The protocol with orcein is particularly simple.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. Cover the section with 5 drops of **reagent A (Potassium permanganate)** + 5 drops of **reagent B (Acid Activation Buffer)** for 10 minutes
3. Wash in distilled water
4. Cover the section with **reagent C (Oxalic Acid)** until it turns white
5. Wash in distilled water
6. Wash in running tap water for 3 minutes
7. Cover the section with **reagent D (Acid Orcein)** for 30 minutes (to highlight HBsAg, incubate for 3 hours)
8. Wash in running tap water for 5 minutes
9. Cover the section with **reagent E (Jenkins reagent)** for 30 seconds
10. Dehydrate quickly, clear and mount with balsam

Results
Elastic fibers and HBsAg: Red-Brown

Preparation	Paraffin section
Control	Liver
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	50 min
Suggested fixatives	Formalin
Critical step	The differentiation with reagent E



Aorta. Red-brown stain of elastic fibers.

HISTOLOGICAL STAINING KIT

AFOG (ACID FUCHSIN ORANGE G) STAIN
code 010307

IVD CE

Description
The Kit supplies reagents for AFOG stain of renal biopsies. It can be used instead of P.A.S.M. staining protocol because of it combines staining capacity of blue aniline, acid fuchsin and Orange G.
Warning: Bouin (or picroformol) is the recommended fixative; if the tissue is fixed with formalin, the muscle turns red instead of green.

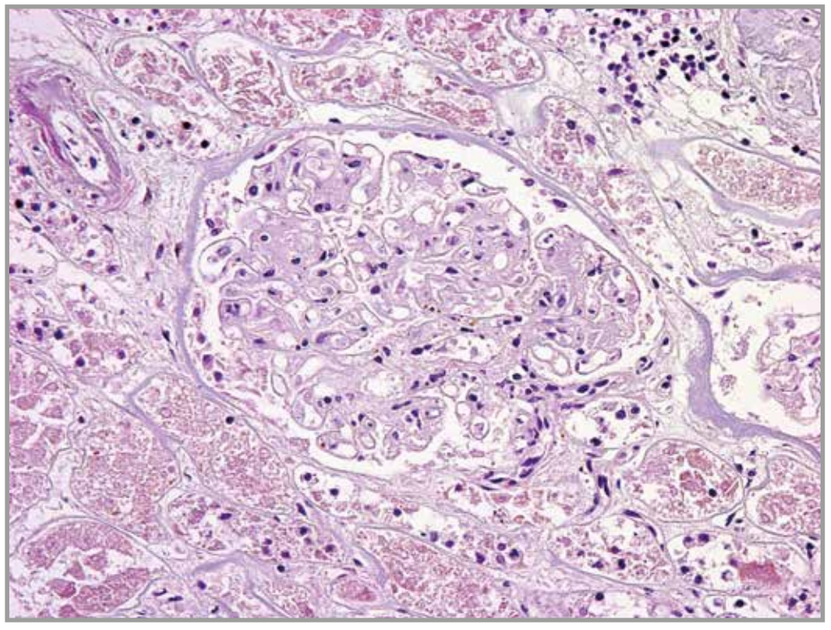
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. **Reagent A (Bouin)** for 3 hours at +56°C. Leave it to cool at room temperature for 10 minutes. Wash in distilled water
3. Put on the section, for 10 minutes, 5 drops of **reagent B (Weigert hematoxylin sol. A)** + 5 drop of **reagent C (Weigert hematoxylin sol. B)**, then wash in running tap water for 5 minutes
4. **Reagent D (Jenkins reagent)** for 4-10 seconds
5. Wash quickly in distilled water
6. **Reagent E (Phosphomolibdic Acid)** for 5 minutes
7. Wash quickly in distilled water
8. **Reagent F (AFOG solution)** for 5-10 minutes
9. Wash in distilled water for 1 minute
10. Dehydrate quickly, clear and mount with balsam

Results

Connective tissue:	Blue
Muscle:	Green (if the specimen is fixed in formalin, Red)
Basal membrane:	Fuchsia
Nuclei:	Black
Fibrin/Erythrocytes:	from Yellow to Red

Preparation	Paraffin section
Control	Kidney
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	3h 40 min
Suggested fixatives	Bouin fixative, Formalin
Critical step	None



Kidney. Renal glomerule stain.

HISTOLOGICAL STAINING KIT

AGNOR
code 010801

IVD CE

Description
The AgNOR Kit is intended for use, by silver impregnation, in histological visualization of the proteins bound to Nucleolar Organizer Region (NOR)

Staining protocol
NOTE: do not use metallic objects, use only distilled water to wash the slides.
The AgNOR staining protocol requires only just cut sections. Do not use polylysine or positively charged slides as they may cause background staining that interferes with preparation reading.
After mounting, keep the slides in a dark place.

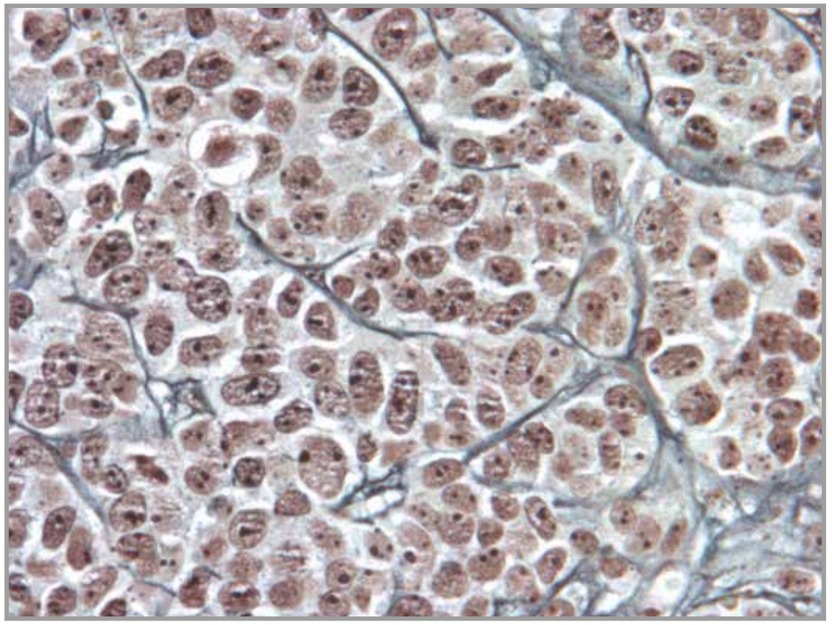
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. Prepare the working solution: 8 ml **reagent A (Gelatine)** + 16 ml **reagent B (Silver nitrate)**. Stir briefly with a glass stick, DO NOT use metallic objects
3. Immerse slides in the solution for 30 minutes at room temperature and in the darkness
4. Drain slides and go to the next step
5. **Reagent C (Fixing solution)** for 1 minute
6. Wash in distilled water for 1 minute
7. Dehydrate, clear and mount with balsam

Results
AgNOR: Black

WARNING: reagents are stored at +4°C/+8°C, we recommend to keep them at room temperature before use for at least 10 minutes. If they are used cold, the reaction speed is significantly reduced.

Preparation	Paraffin section
Control	Tonsil
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	35 min
Suggested fixatives	Formalin
Critical step	Do not use metallic objects. The working solution deteriorates quickly, use soon after preparation. Protect the reagent from light by covering the jar with an aluminum foil. Use only just cut sections.



Mouse breast with cancer. Black stain of NOR regions.

HISTOLOGICAL STAINING KIT

ALCIAN BLUE PH 0.2 acc. DORLING
code 010203

ALCIAN BLUE PH 0.5 acc. DORLING
code 010204

ALCIAN BLUE PH 1.0 acc. DORLING
code 010205

ALCIAN BLUE PH 1.5 acc. DORLING
code 010206

IVD CE

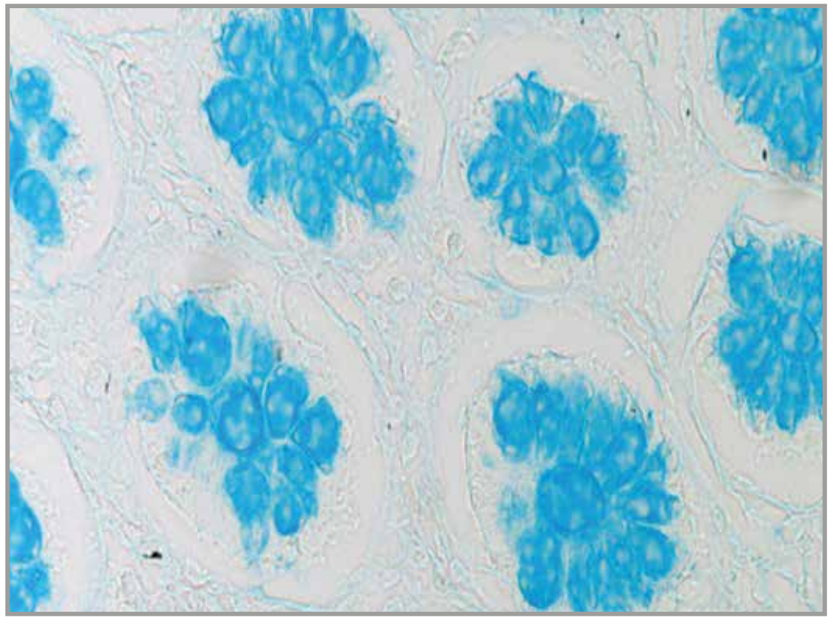
Description
The Kits are intended for use in histological visualization of mucins. The treatment with acid buffer allows a greater staining specificity. The mucins are composed by glycolproteins and, depending on pH, the acid groups will be more or less differentiated. In particular: Alcian Blue pH 0.2 and Alcian Blue pH 0.5 allow to show greatly sulphated mucins. Alcian Blue pH 1.0 allows to show the weakly and greatly sulphated groups. Alcian Blue pH 1.5 allows to show residues of hyaluronic and sialic acid.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. **Reagent A (Acid buffer)** for 10-15 minutes
3. Drain slide and go to the next step
4. **Reagent B (Alcian Blue)** for 30 minutes
5. Drain slide and allow to dry in the open air
6. Clear and mount with balsam

Results
Mucins: Blue-Turquoise
Background: Colorless

Preparation	Paraffin section
Control	Appendix, colon
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	40 – 45 min
Suggested fixatives	Formalin
Critical step	pH of solutions



Intestine, colon. Blue stain of mucins, absent counterstaining.

HISTOLOGICAL STAINING KIT

ALCIAN BLUE PH 2.5 acc. DORLING
code 010207

IVD CE

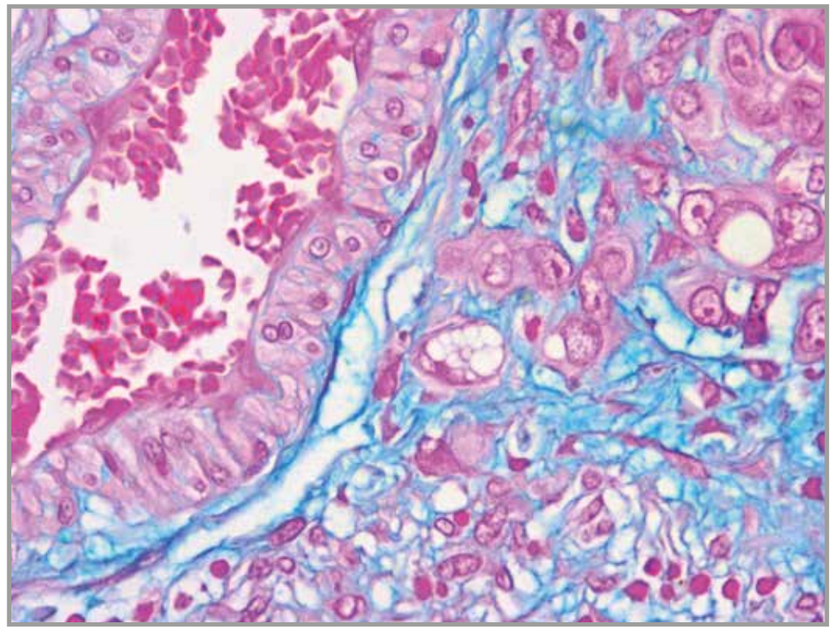
Description
The Kit supplies reagents to visualize mucins with Alcian Blue. In particular the solution pH 2.5 stains acid mucins but doesn't visualize the sulphated mucins. A washing in which the solution has the same pH than stain one, provides a greater reaction specificity. Nuclei and counterstains are showed in red.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. **Reagent A (Alcian Blue pH 2.5)** for 45 minutes
3. Drain slide and go to the next step
4. **Reagent B (Sodium Tetraborate)** for 10 minutes
5. Distilled water for 5 minutes
6. **Reagent C (Kernechtrot)** for 5 minutes
7. Distilled water for 2 minutes
8. Dehydrate quickly, clear and mount with balsam

Results
Mucins: Blue-Turquoise
Nuclei: Red

Preparation	Paraffin section
Control	Appendix, colon
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	1h 10 min
Suggested fixatives	Formalin
Critical step	Solution pH



Omentum with invasive carcinoma. Blue stain of mucins, counterstaining in red.

HISTOLOGICAL STAINING KIT

ALCIAN BLUE PH 3.1 acc. DORLING
code 010208

IVD CE

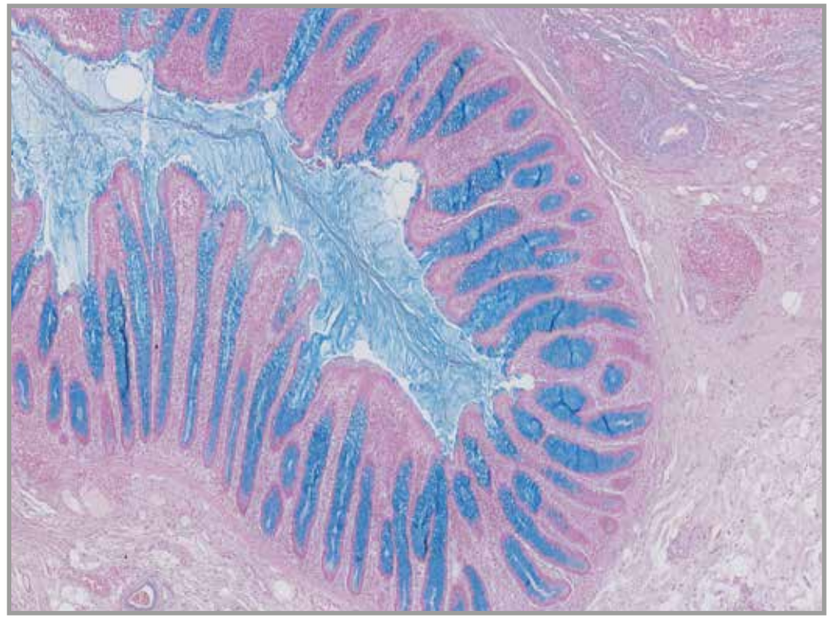
Description
The Kit supplies reagents to visualize mucins with Alcian Blue. In particular the solution pH 3.1 stains the acid mucopolysaccharides. A washing in which the solution has the same pH than stain ones provides a greater reaction specificity. Nuclei counterstaining with Kernechtrot.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. **Reagent A (Alcian Blue pH 3.1)** for 45 minutes
3. Drain slide and go to the next step
4. **Reagent B (Sodium Tetraborate)** for 10 minutes
5. Distilled water for 15 minutes
6. **Reagent C (Kernechtrot)** for 5 minutes
5. Distilled water for 2 minutes
7. Dehydrate quickly, clear and mount with balsam

Results
Mucins: Blue-Turquoise
Nuclei: Red

Preparation	Paraffin section
Control	Appendix, colon
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	1h 15 min
Suggested fixatives	Formalin
Critical step	Solution pH



Intestine, colon. Blue stain of mucins, nuclei counterstaining in red.

HISTOLOGICAL STAINING KIT

ALCIAN BLUE PH 2.5 - P.A.S. acc. MOWRY
code 010209

IVD CE

Description
The Kit supplies reagents for Alcian Blue pH 2.5 and P.A.S. stains to show the acid mucins, glycoproteins and glycogen on the same section. The P.A.S. (Periodic Acid Schiff) stain visualises the glycogen and glycoproteins, many tissues can be P.A.S. positive. The Schiff reagent is a watery colorless solution and is used bound to the periodic acid to show the aldehyde groups. The nuclear contrast is obtained with Mayer hematoxylin.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. **Reagent A (Alcian Blue)** pH 2.5 for 30 minutes. Drain slide and, without washing the section, go to the next step
3. **Reagent B (Sodium Tetraborate)** for 10 minutes
4. Wash in cold running tap water for 5 minutes. Wash in distilled water for 1-2 minutes
5. **Reagent C (Periodic Acid)** for 5 minutes. Wash in distilled water for 2 minutes
6. **Reagent D (Schiff Reagent)** for 30 minutes. Wash in distilled water for 2 minutes
7. Working solution: 80 ml of distilled water + 10 drops of **reagent E (Potassium Metabisolfite)** + 10 drops of **reagent F (Hydrochloric Acid)**. Immerse the slides in the working solution for 10 minutes.
8. Wash in distilled water for 1 minute
9. **Reagent G (Mayer Hematoxylin)** for 1 minute
10. Running tap water for 1 minute
11. Dehydrate quickly, clear and mount with balsam

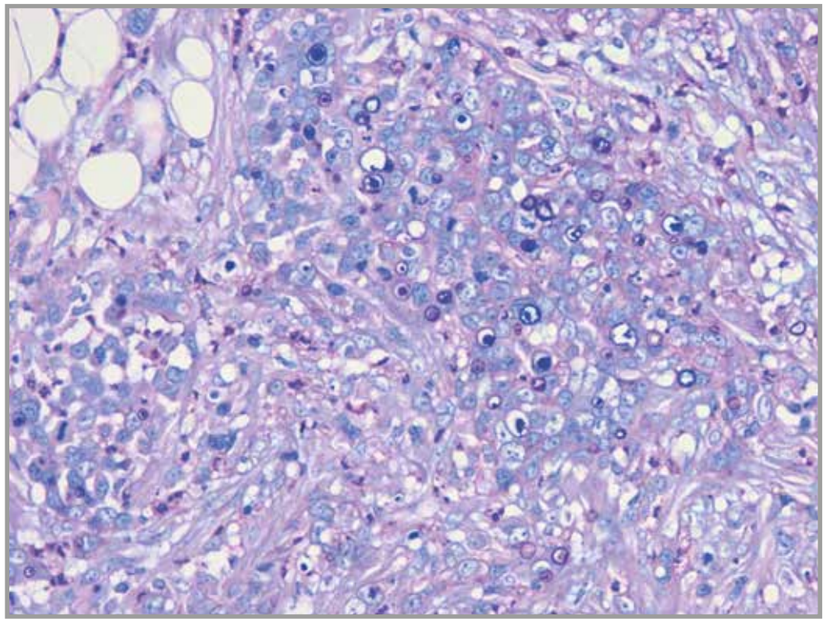
WARNING: we recommend to keep **reagent D (Schiff reagent)** at room temperature for at least 10 minute before the use.

Results

Mucins:	Blue-Turquoise
Positive P.A.S. substances:	Magenta
Nuclei:	Blue-Violet
Epithelial mucins and cartilages:	Purple-Dark blue

WARNING: reagents are stored at +4°C/+8°C, we recommend to keep them at room temperature before use for at least 10 minutes. If they are used cold, the reaction speed is significantly reduced and it is necessary to increase incubation times.

Preparation	Paraffin section
Control	Appendix, colon
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	1h 40 min
Suggested fixatives	Formalin
Critical step	Solution pH, reagent temperature



Omentum with invasive carcinoma. Mucin stain in blue, P.A.S. positive substances in magenta. Nuclei blue counterstaining.

HISTOLOGICAL STAINING KIT

ALCIAN YELLOW-TOLUIDINE BLUE
code 010269

IVD CE

Description
The Kit supplies reagents to show *Helicobacter pylori* on gastric tissue. Alcian yellow-Toluidine blue stain can be used instead Giemsa stain because of bacteria are particularly visible on the yellow background of gastric mucins.

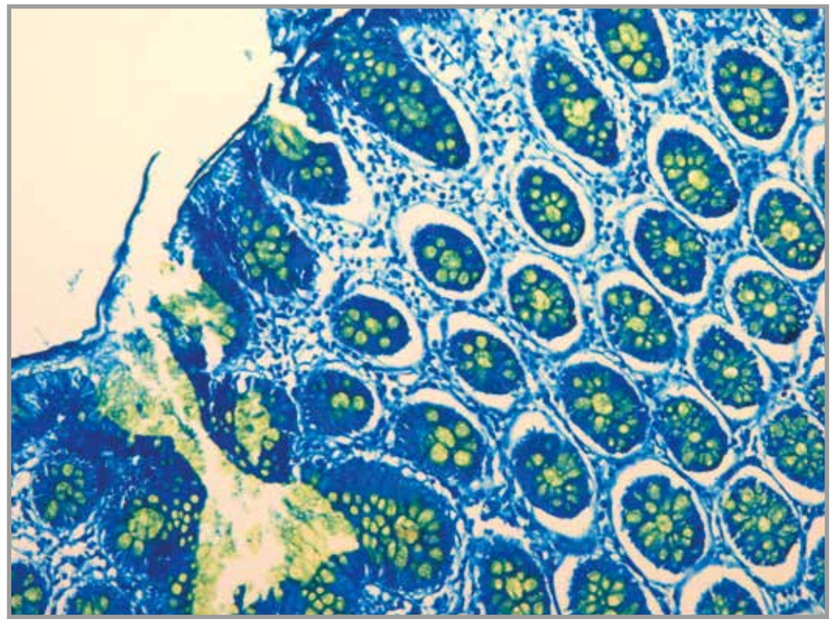
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. **Reagent A (Periodic Acid)** for 10 minutes
3. Wash in running tap water
4. **Reagent B (Potassium Metabisulfite)** for 5 minutes
5. Wash in running tap water
6. **Reagent C (Alcian Yellow)** for 15 minutes
7. Wash in running tap water
8. **Reagent D (Toluidine Blue)** for 5 minutes
9. Wash in running tap water
10. Dehydrate quickly, clear and mount with balsam

Results

Helicobacter pylori:	Dark blue
Mucins:	Yellow
Surrounding tissue:	Light blue

Preparation	Paraffin sections
Control	Stomach (recorded case of *Helicobacter Pylori*)
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	35 min
Suggested fixatives	Formalin
Critical step	None



Stomach. *Helicobacter Pylori* in dark blue. Nuclei and cytoplasm in blue. Yellow mucins.

HISTOLOGICAL STAINING KIT

AZAN TRICHROME
code 010212

IVD CE

Description
The Azan trichrome is a version of Mallory trichrome for connective tissue staining. The Kit is intended for use in histological visualization of fibers, glial fibers, collagen, glomerular stroma and erythrocytes on the same sections. The staining protocol is suitable for hypophysis staining too.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

Pour **reagents A**, **D** and **E** in 3 vertical Choplin jars. Incubate **reagent A** at +56°C.

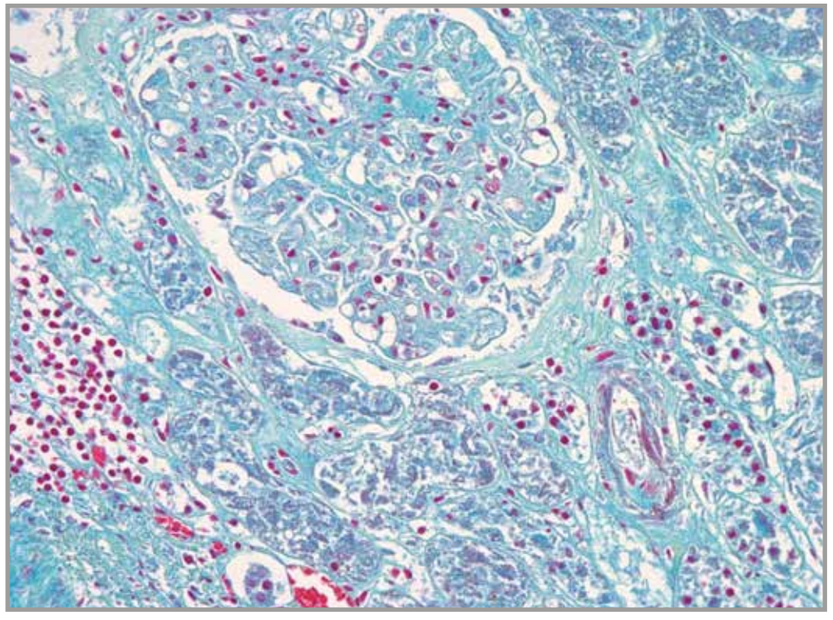
1. Deparaffinize and hydrate section to distilled water
2. Incubate slides in **reagent A (Azocarmine)** for 45 minutes at +56°C
3. Leave it cool at room temperature for at least 10 minutes. Wash in running tap water the excess stain
4. **Reagent B (Blue aniline)** for 1 minute
5. Drain slide and go to the next step
6. **Reagent C (Differentiation solution)** for 1 minute. Wash quickly in distilled water
7. Immerse slides in **reagent D (Phosphotungstic Acid)** for 60 minutes. Drain slide and go to the next step
8. Immerse slide in **reagent E (Mallory solution)** for 60 minutes
9. Quick step in ethylic alcohol 95°
10. Complete dehydration and clear
11. Mount with balsam

Warning: The **reagent A** can be used again without filtering. **Reagents D** and **E** can be used again after filtering.

Results
Nuclei, Erythrocytes: Red
Muscle: Orange
Collagen fibers: Bright light blue

Hypophysis
Cytoplasmatic granules of hypophysis delta cells: Light blue
Acidophil granules of hypophysis: Red

Preparation	Paraffin section
Control	Kidney
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	3h
Suggested fixatives	Formalin
Critical step	Steps at +56°C



Kidney. Blue stain of glomerules. Erythrocytes in red.

HISTOLOGICAL STAINING KIT

CONGO RED
code 010214

IVD CE

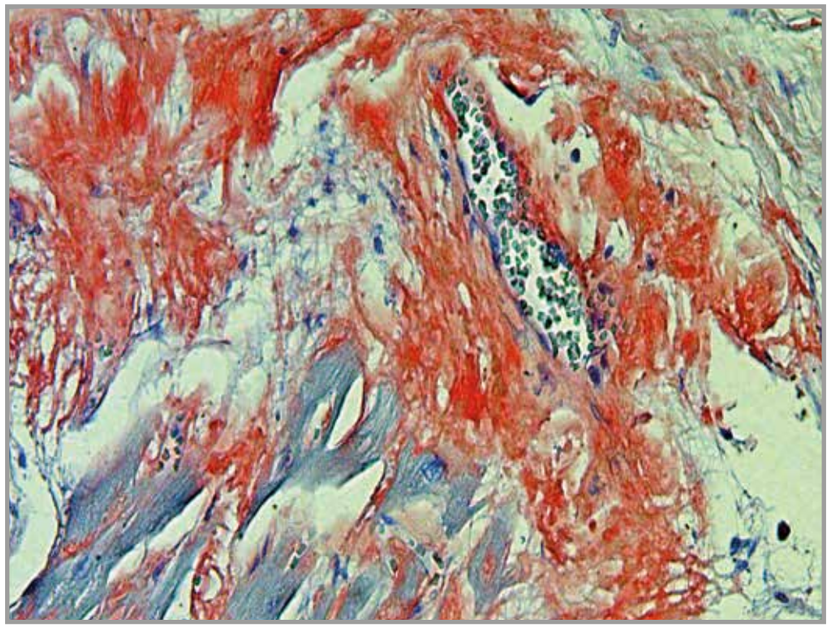
Description
The Kit is intended for use in histological visualization of amyloid (insoluble protein with reduced molecular weight).
The amyloid takes a particular red stain and green birefringence under polarized light.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. **Reagent A (Congo Red)** for 30 minutes
3. Drain the slide and go to the next step
4. **Reagent B (Lithium Carbonate)** for 10 minutes
5. Wash quickly in distilled water
6. **Reagent C (Alcoholic Differentiation Buffer)** for 15-30 seconds
7. Wash in running tap water for 5 minutes
8. **Reagent D (Mayer Hematoxylin)** for 5 minutes
9. Running tap water for at least 10 minutes
10. Dehydrate quickly, clear and mount with balsam

Results
Amyloid: from Pink to Red
Nuclei: Blue-Violet

Preparation	Paraffin section
Control	Positive case (ex. amyloidosis)
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	1h
Suggested fixatives	Formalin
Critical step	To highlight, with polarized light, cut section with thickness of at least 10 µm



Connective tissue. Red stain of amyloid deposits.

HISTOLOGICAL STAINING KIT

DANE TRICHROME
code 010215

IVD CE

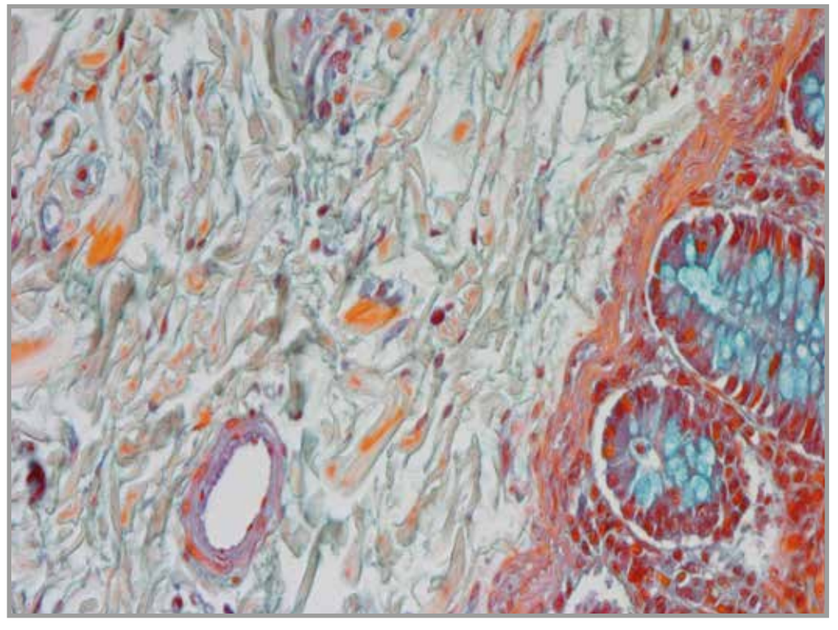
Description
The Kit is intended for use in histological simultaneously visualization of prekeratins, keratins and acid mucins.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. **Reagent A (Mayer Hematoxylin)** for 10 minutes
3. Running tap water for 5 minutes
4. **Reagent B (Floxin)** for 3 minutes
5. Running tap water until the section looses completely the stain
6. **Reagent C (Alcian Blue)** for 5 minutes
7. Running tap water for 5 minutes
8. **Reagent D (Orange G)** for 13-15 minutes
9. Distilled water for 1 minute
10. Dehydrate quickly, clear and mount with balsam

Results
Mucins: Blue
Keratin: from Orange to Red
Nuclei: Brown-Red

Preparation	Paraffin section
Control	Skin
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	45 – 50 min
Suggested fixatives	Formalin
Critical step	None



Intestine, colon. Blue stain of mucins.

HISTOLOGICAL STAINING KIT

DIASTASE BUFFER
code 010216

IVD CE

Description
The Kit supplies the Diastase solution that contains an enzyme that hydrolyzes the glycogen present in the tissue. The following P.A.S. staining will visualize other P.A.S. positive substances such as neutral epithelial mucins.
The Kit provides all the necessary for the pre-treatment of the sections but not the reagents for the P.A.S. staining.

Staining protocol
To avoid section excessive drying, use an incubator box.

Diastase Buffer: pour the contents of one capsule of **reagent B (Amylase)** in a bottle of **reagent A (Phosphate Buffer)** and stir until the complete powder melting, do not filter the solution.

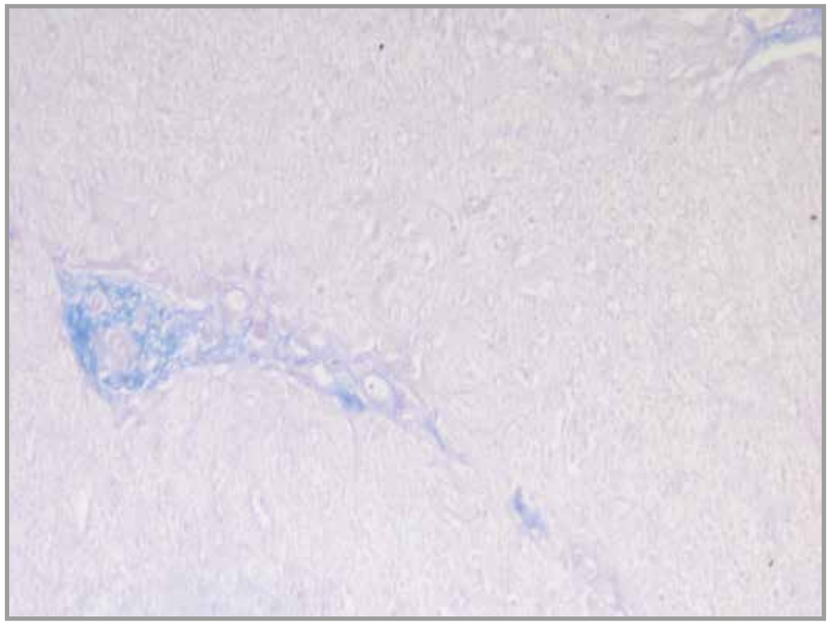
WARNING: Once in solution, the enzyme isn't stable. Store the solution at +4°C and use it after 48 hours from the preparation.
Prepare 2 sections of which only one will be treated with diastase buffer.

1. Deparaffinize and hydrate to distilled water
2. Immerse slide in **Diastase Buffer** solution
3. Incubate for 1 hour at room temperature or 30 minutes in oven at +40°C
4. Wash in distilled water
5. Proceed with P.A.S. staining on both sections

Results
The comparison between the two sections allows to visualize areas of response to P.A.S. stain due to the real presence of glycogen.
Section treated with buffer diastase: P.A.S. + not due to the presence of glycogen.
Section not treated with buffer diastase: P.A.S. + for both glycogen and reactive substances.

WARNING: reagents are stored at +4°C/+8°C, we recommend to keep them at room temperature for at last 10 minutes before the use.
If they are used cold, the reaction speed is significantly reduced.

Preparation	Paraffin section
Control	Section for P.A.S. stain (liver)
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	1h
Suggested fixatives	Formalin
Critical step	Reagent temperature. Diastase solution isn't stable, use within 24 - 48 h and store at +4°C/+8°C



Liver. Negative P.A.S. stain for glycogen after treatment with diastase.

CYTOLOGICAL STAINING KIT

FAST QUICK - M.G.G. RAPID
code 010253

IVD CE

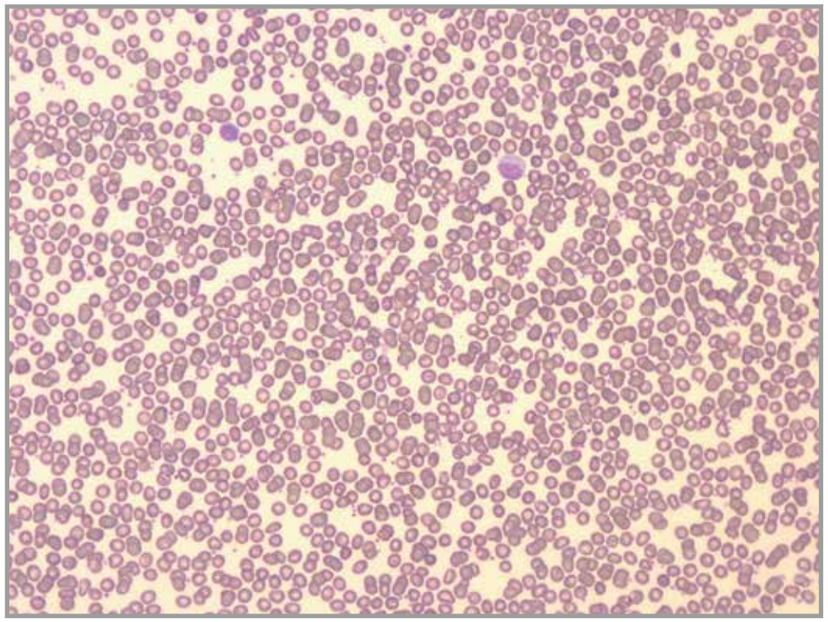
Description
The Kit supplies reagents for the fast stain of blood smears. The M.G.G. (May Grunwald Giemsa) stain allows to visualize the different kind of blood cells.

Staining protocol
1. Dry the smear in the air
2. Immerse the slide for 5 times for 1 second each in **reagent A**. After immersion, wait up to complete dripping of excess liquid
3. Immerse the slide for 5 times for 1 second each in **reagent B**. After immersion, wait up to complete dripping of excess liquid
4. Immerse the slide 3-5 times for 1 second each in **reagent C**. After immersion, wait up to complete dripping of excess liquid
5. Wash with spring water
6. Dry in the air (do not use heat sources, ovens or plates)
7. Clear and mount with balsam

Results

Nuclei:	Violaceous Red, Pink
Basophil cytoplasm:	from light Blue to dark Blue
Acidophil cytoplasm:	from light to rosy Red
Polychromatophilic cytoplasm:	from Grayish to Violaceous
Acidophil granules:	Orange
Neutrophil granules:	Brown-dark Pink
Basophil granules:	Dark Violet
Azurophil granules:	from Purple to Purple Violet

Preparation	Blood smear
Control	Peripheral blood smear
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	20-25 seconds
Suggested fixatives	Non-foreseen
Critical step	None



Blood smear. Stain to show the different cell components.

HISTOLOGICAL STAINING KIT

FAST QUICK HEMATOXYLIN - EOSIN
code 010263

IVD CE

Description
The Kit supplies reagents for the fast Hematoxylin-Eosin stain on frozen sections.

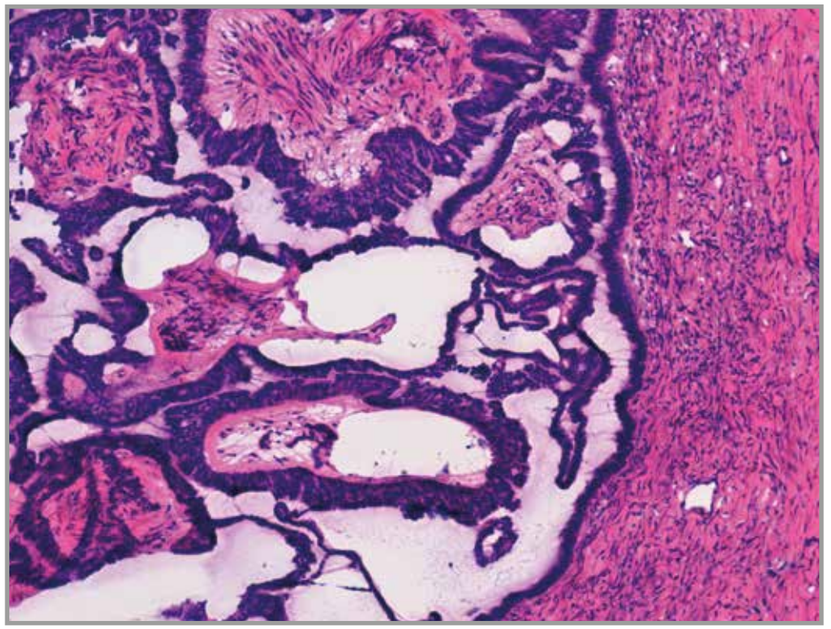
Staining protocol
Pour reagents in histology jars and immerse slides.

1. Prepare the toning solution: 10 drops of **reagent B (Basic buffer)** in 100 ml of distilled water
2. **Reagent A** (hematoxylin) for 50 seconds
3. Wash the slide in a bowl with distilled water or spring water
4. Immerse now the slide in the solution: 5 quick immersions
5. **Reagent C** (alcoholic eosin) for 30 seconds
6. 5 quick immersions in ethyl alcohol 50°
7. 5 quick immersions in absolute ethyl alcohol
8. Clarify and mount in balsam

WARNING: **reagents B** and **C** are re-usable and they don't need filtration.

Results
Cytoplasm: Pink-Orange
Nuclei: Blue-Violet

Preparation	Cryostat section
Control	Any tissue
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	5 min
Suggested fixatives	Non-foreseen
Critical step	Avoid slide drying



Omental metastases of intestine adenocarcinoma.

HISTOLOGICAL STAINING KIT

FEULGEN AND ROSSENBECK
code 010219

IVD CE

Description
The Kit supplies the reagents necessary to show DNA with Schiff reagent according to Feulgen and Rossenbeck staining protocol. The specimen is treated with hydrochloric acid which removes the purine bases and makes available the aldehyde groups to Schiff reagent. This reaction is specific for DNA.
We recommend to analyse the slides the same day of the staining.

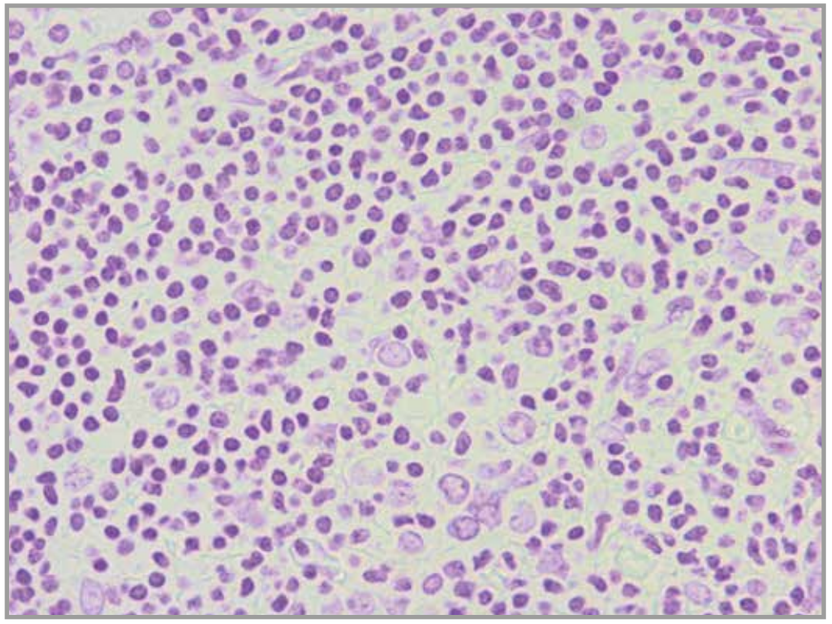
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water (for histological specimens)
   Running tap water for 5 minutes (for cytological specimens)
2. **Reagent A (Hydrochloric Acid)** for 10 minutes
3. Wash the section in distilled water
4. **Reagent B (Schiff reagent)** for at least 5 minutes (till the section turns magenta)
5. Drain reagent in excess and go to the next step without washing the section
6. **Reagent C (Metabisolphite Potassium)** for 2 minutes
7. Drain reagent in excess and go to the next step without washing the section
8. **Reagent D (Hydrochloric Acid)** for 3 minutes
9. Wash in running tap water
10. Dehydrate quickly, clear and mount with balsam

Results
DNA: Red magenta
Background: Colorless

WARNING: we recommend to not use Bouin's fixative, as acid fixatives may interfere with hydrolysis process.
If treatment with hydrochloric acid is too long, it may cause the complete DNA hydrolysis with consequent reduction of reaction sensitivity and appearance of possible false negatives.
Warning: the decalcification process interferes with the staining so the bony tissue is not usually indicated for this kind of investigation.
We recommend leaving the **reagent D (Schiff Reagent)** at room temperature for at least 10 minutes before use it. If used cold, the reaction speed decreases considerably.

Preparation	Paraffin section
Control	Unknown control tissue
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	25 min
Suggested fixatives	Formalin
Critical step	Do not use acid fixatives. Do not prolong treatment with reagent A



Lymphnode. Nuclei magenta staining. Absent counterstaining.

HISTOLOGICAL STAINING KIT

FOUCHET-VAN GIESON acc. KUTLICK
code 010220

IVD CE

Description
The Kit is intended for use in histological simultaneously visualization of bilirubin, connective tissue and collagen. Bilirubin is a yellow-brown pigment resulting from hemoglobin catabolism. It turns green due to oxidation induced by Fouchet solution. The counterstaining with Van Gieson Picrofuchsin allows to differentiate the connective tissue and collagen.

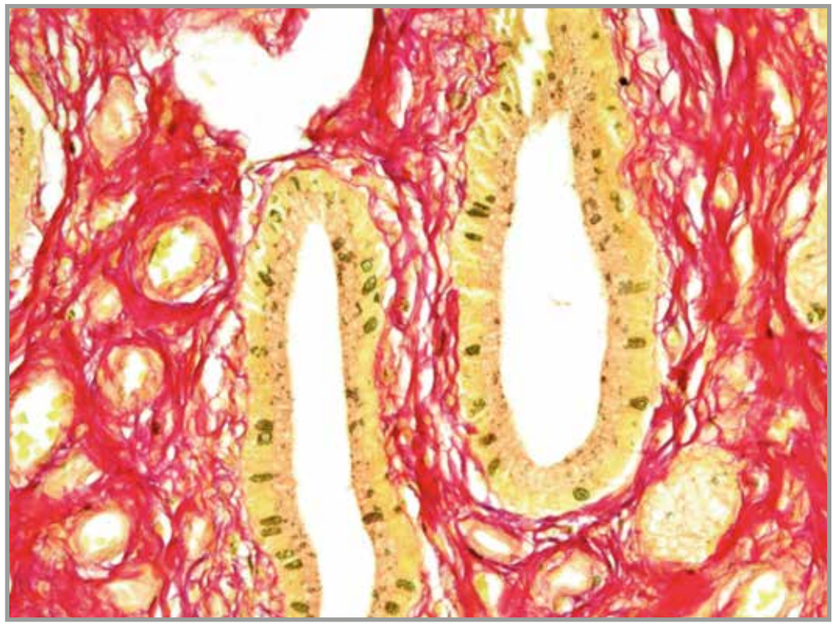
Staining protocol
Drain reagents directly on section in a way to cover it completely.

1. Deparaffinize and hydrate section to distilled water
2. Cover sections with 10 drops of **reagent A** + 10 drops of **reagent B**, incubate for 5 minutes
3. Wash with distilled water for 2 minutes
4. Cover the sections with **reagent C** for 5 minutes
5. Drop the excess liquid and pad with filter paper
6. Dry in the air
7. Xylol or substitutes, mount

Results

Bilirubin:	Green
Connective tissue:	Red
Collagen/Muscle:	Yellow

Preparation	Paraffin section
Control	Liver with biliar statis
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	15 min
Suggested fixatives	Formalin
Critical step	If alcohols are used for preparation dehydration, the final staining can turn weak



Cholecyst. Bilirubin in green. Counterstaining with Van Gieson stain.

HISTOLOGICAL STAINING KIT

GOLDNER TRICHROME (MASSON'S TRICHROME WITH LIGHT GREEN)

code 010224

IVD CE

Description
The Kit is intended for use in histological visualization of connective tissue, collagen, reticular fibers and muscle fibers. The procedure gives a particular green staining of collagen fibers.

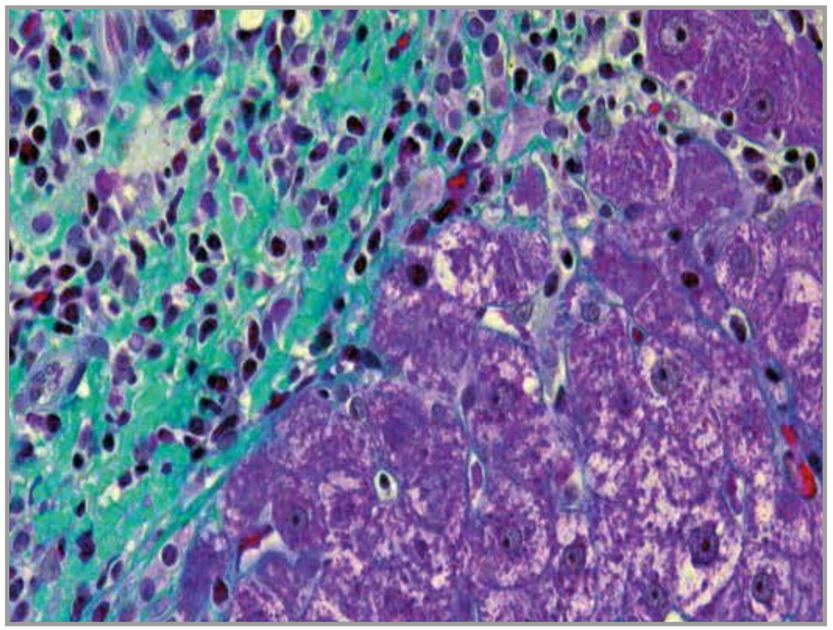
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. 5 drops of **reagent A (Weigert hematoxylin sol. A)** + 5 drops of **reagent B (Weigert hematoxylin sol. B)** for 10 minutes
3. Running tap water for at least 5 minutes
4. **Reagent C (Picric Acid)** for 4 minutes
5. Wash in distilled water for 30 seconds
6. **Reagent D (Ponceau Fuchsin)** for 4 minutes
7. Drain section and go to the next step
8. **Reagent E (Phosphomolibdic Acid)** for 5 minutes. Drain slide and go to the next step
9. **Reagent F (Light Green)** for 5 minutes
10. Dehydrate quickly, clear and mount in balsam

Results

Nuclei:	Black
Muscle fibers, keratin, cytoplasm:	Bright Red
Collagen, mucus:	Green
Erythrocytes:	Yellow-Orange

Preparation	Paraffin section
Control	Liver, stomach
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	40 min
Suggested fixatives	Formalin
Critical step	None



Liver. Green stain of connective tissue.

HISTOLOGICAL STAINING KIT

GOMORI'S TRICHROME
code 010302

IVD CE

Description
The Kit allows the analysis of collagen fibers in liver and kidney tissue. Suitable to visualize the ragged red fibers present in many mitochondrial myopathies.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

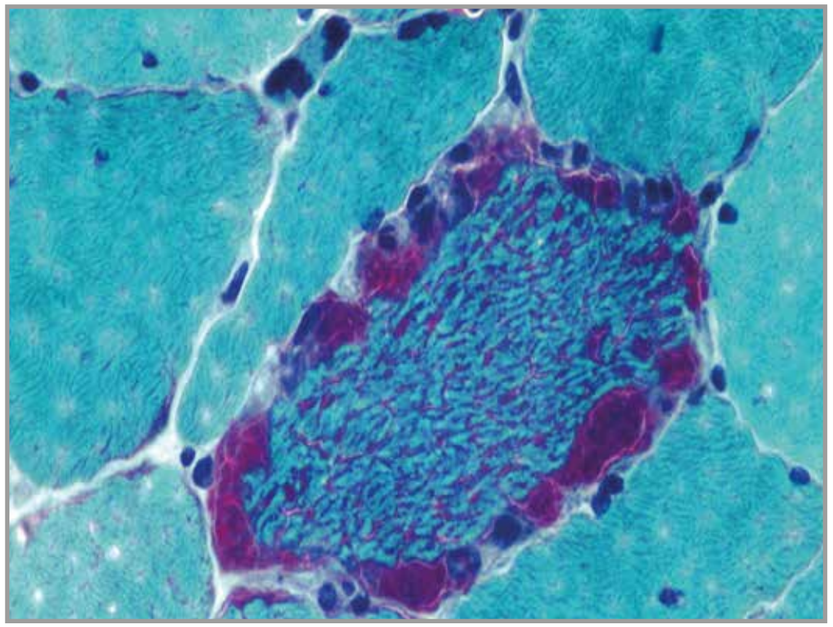
1. Hydrate sections up to distilled water
2. **Reagent A (Mayer hematoxylin)** for 45 seconds
3. Wash in running tap water until nuclei change stain
4. Wash in distilled water for 30 seconds
5. **Reagent B (Gomori Stain)** for 10 minutes
6. Wash in distilled water
7. **Reagent C (Acid Buffer)** for 15 seconds
8. Drain slide and go to the next step
9. **Reagent D (Differentiation solution)** for 20 seconds
10. Wash in distilled water
11. Dehydrate quickly, clear and mount in balsam

Results

Myofibrils:	Green (*)
Intermyofibrillar material:	Red
Connective tissue:	Bright Green
Nuclei:	Blue-Violet

(*) if the tissue is fixed in formalin, the muscle turns in red

Preparation	Cryostat section
Control	Muscle
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	15 min
Suggested fixatives	Non-foressen
Critical step	Non-suitable for tissue embedded in paraffin



Frozen tissue. Pathological muscle.

HISTOLOGICAL STAINING KIT

GRAM FOR HISTOLOGICAL SECTIONS
code 010221

IVD CE

Description
The Kit supplies reagents to visualize the Gram+ bacteria with Gram stain on paraffin sections. According to stain, the bacteria are classified in Gram positive (Gram+) and Gram negative (Gram-) showing some properties of the cell wall present in bacteria: the Gram+ are characterized by a cell wall, rich in sugars and aminoacids. The Gram- have a thin cell wall, rich in lipopolysaccharides and lipoproteins. With this staining, it is possible to show the *Pneumocystis carinii* protozoan.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

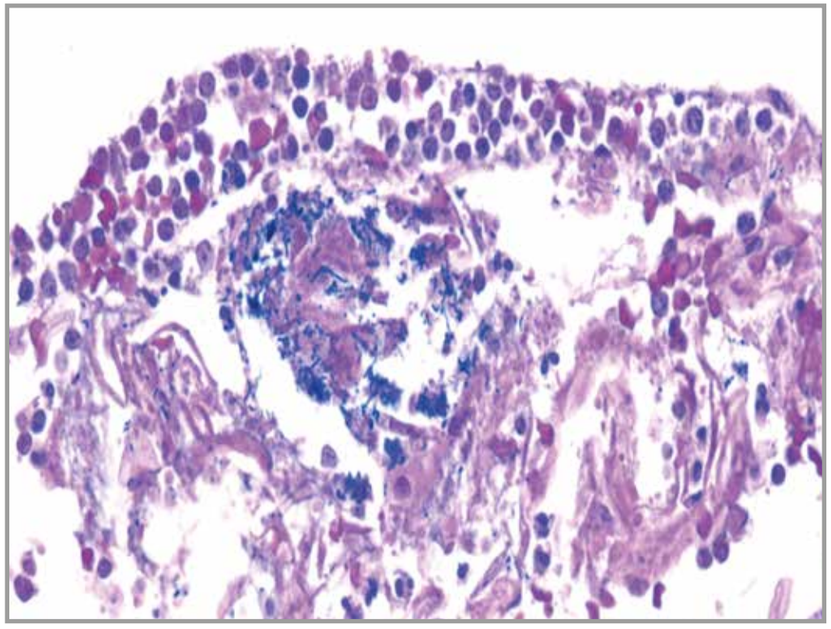
1. Deparaffinize and hydrate section to distilled water
2. **Reagent A (Mayer Hematoxylin)** for 5 minutes
3. Running tap water for 5 minutes
4. Immerse slides for 15 minutes in **Reagent B (Floxin B)** pre-heated at +56°/+58°C
5. Wash in distilled water
6. **Reagent C (Crystal Violet)** for 3 minutes
7. Drain reagent in excess and go to the next step
8. **Reagent D (Iodium-Iodide Solution)** for 3 minutes
9. Wash in distilled water for 2 minutes. Leave the section to dry
10. **Reagent E (Xylene-Aniline)** in immersion for 1 minute
11. **Reagent F (Xylene-Aniline)** in immersion for 1 minute
12. Clear and mount in balsam

WARNING: **reagent B** can be used again after filtration.

Results

Pneumocystis Carinii, mycosis and Gram+ bacteria:	Violet
Nuclei:	Blue-Violet
Paneth cell granules, keratoialine e keratin:	Blue
Cytoplasm:	from Pink to Red

Preparation	Paraffin section
Control	Tissue with reported Gram+ infection
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	40 min
Suggested fixatives	Formalin
Critical step	Reagent temperature



Tonsil. Bacteria blue stain.

HISTOLOGICAL STAINING KIT

GRIMELIUS
code 010222

IVD CE

Description
The Kit is intended for use in histological visualization of pancreas alpha cells. The Grimelius stain is also designed for demonstrating cells secreting argyrophilic substances such as noradrenaline, serotonin, lipofuchsin.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

WARNING: Use a oven at +60°C for the first impregnation. The second impregnation occurs at room temperature.

1. Deparaffinize and hydrate section to distilled water
2. Working silver nitrate solution: 40 ml of distilled water + 2 ml **reagent A (Silver Nitrate)** + 4 ml **reagent B (Acetate buffer)**. Preserve 1-2 ml of this solution for the second impregnation. Protect reagent from light

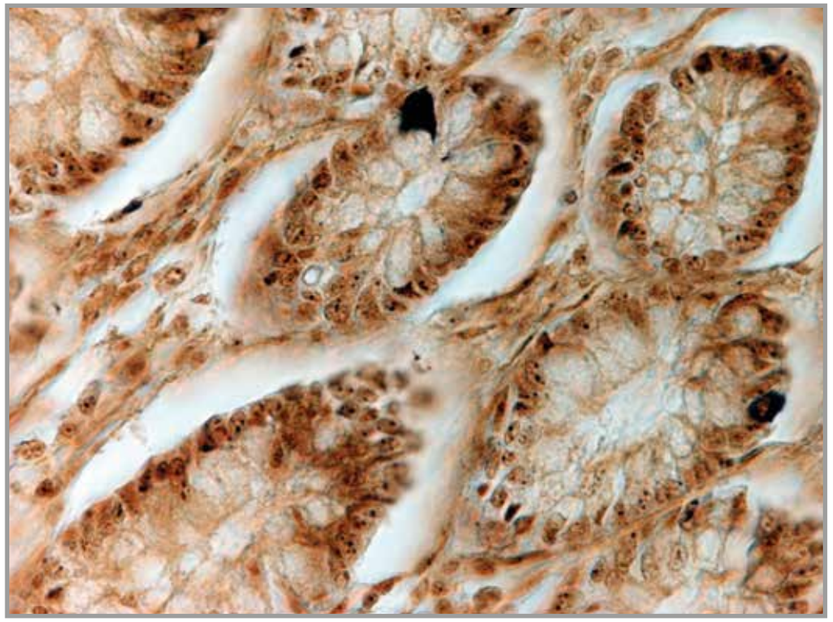
FIRST IMPREGNATION:
3. Cover section with working solution of silver nitrate (STEP 2) and incubate in oven at +60°C in the darkness for 3 hours. Leave it cool at room temperature
4. Reducing solution: melt **reagent C (Reducing powder)** in 25 ml of distilled water. Stir till the complete powder melting. Preserve 1-2 ml of this solution for the second impregnation
5. Immerse slides in the reducing solution (STEP 4) and leave in oven at +60°C in the darkness for 5 minutes. Leave it cool at room temperature. Wash in distilled water for 3 minutes

SECOND IMPREGNATION:
6. Cover section with working solution of silver nitrate (STEP 2) at room temperature for 10 minutes
7. Drain the reagent from the slide and go to the next step
8. Cover section with working solution of silver nitrate (STEP 4) at room temperature for 5 minutes
9. Wash in distilled water for 3 minutes
10. **Reagent D (Fixing Solution)** for 2 minutes
11. Wash in distilled water
12. Dehydrate quickly, clear and mount with balsam

Results
Argyrophilic granules: from light Brown to Black

WARNING: reagents are stored at +4°C/+8°C, we recommend to keep them at room temperature before use for at least 10 minutes. If they are used cold, the reaction speed is significantly reduced.

Preparation	Paraffin section
Control	Pancreas or intestine
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	3h 40 min
Suggested fixatives	Formalin
Critical step	Reagent temperature. Don not use metallic objects. For the first impregnation, protect specimen from light covering the jar



Intestine. Brown-black stain of argyrophilic substances.

HISTOLOGICAL STAINING KIT

GROCOTT acc. CALLARD
code 010223

IVD CE

Description
The Kit is designed for demonstrating fungi in tissue sections. To prevent the tissue section drying, the staining protocol foresees the immersion of slides in silver-methenamine solution.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

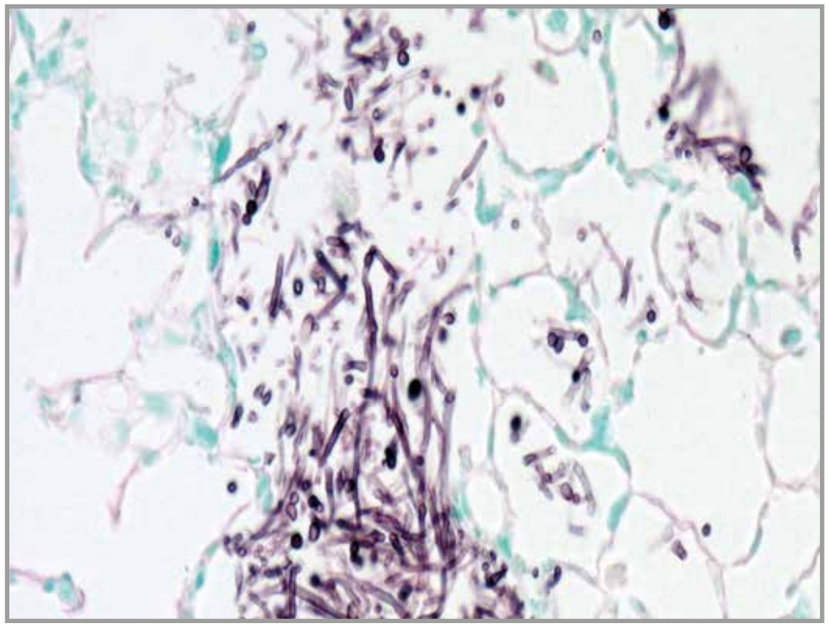
1. Prepare the methenamine silver working solution: 15 drops of **reagent C (Silver Nitrate)** + 30 drops of **reagent D (Methenamine)** + 20 drops of **reagent E (Sodium Tetraborate)** + 40 ml of distilled water. Stir with a glass stick, DO NOT use metallic objexts. Preheat at +56°C
2. Deparaffinize and hydrate section to distilled water
3. **Reagent A (Chromic Acid)** for 20 minutes. Wash in distilled water
4. **Reagent B (Metabisulfite Potassium)** for 1 minute. Wash in running tap water for 1 minute
5. Wash in distilled water
6. Immerse slides in the methenamine silver working solution (STEP 1) and incubate at +56°C for 1 hour. Control at microscope the staining degree. If necessary prolong incubation times (fungi turn dark brown on colorless background)
7. Leave slides cool at room temperature for 5 minutes. Wash in distilled water
8. **Reagent F (Gold chloride)** for 3 minutes. Wash in distilled water
9. **Reagent G (Sodium thiosulfate)** for 5 minutes. Wash in distilled water
10. **Reagent H (Light green)** for 1 minute
11. Wash quickly in distilled water
12. Dehydrate quickly, clear and mount in balsam

Results

Basal membranes, glycogen, micete capsule:	Black
Mucins e glycogen:	Grey-Black
Erythrocytes:	Yellow
Background:	Green

WARNING: reagents are stored at +4°C/+8°C, we recommend to keep them at room temperature before use for at least 10 minutes. If they are used cold, the reaction speed is significantly reduced.

Preparation	Paraffin section
Control	Fungal infection
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	1h 40 min
Suggested fixatives	Formalin
Critical step	Reagent temperature. Don not use metallic objects. Wash in distilled water



Mouse lung. Fungi black stain. Green counterstaining.

HISTOLOGICAL STAINING KIT

HALE REACTION
code 010312

IVD CE

Description
The Kit is designed for demonstrating colloidal iron and polysaccharides acids, such as ialuronic acid in histological sections.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. Cover section with 10 drops of **reagent A (Colloidal iron solution)** and 10 drops of **reagent B (Acid solution)** for 10 minutes
3. Wash several times in distilled water
4. Prepare 100 ml of potassium ferrocyanide solution: melt **reagent C (Porassium ferro cyanide)** in 80 ml of distilled water, then add 20 ml of **reagent D (Chloride acid)**. DO NOT use metallic objects.
5. Immerse slides in potassium ferrocyanide solution (STEP 4) for 10 minutes
6. Wash more times in distilled water
7. Cover section with **reagent E (Kernechtrot)** for 5 minutes
8. Wash quickly in distilled water
9. Dehydrate quickly, clear and mount with balsam

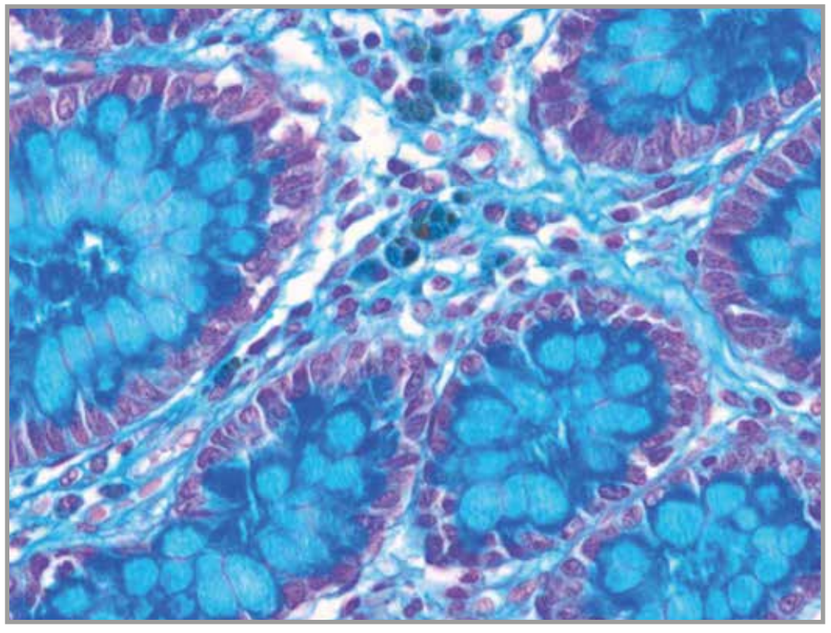
WARNING: the potassium ferrocyanide solution should be fresh when used. Use again the solution could bring to false positives.
We recommend to use a positive control tissue.

Results

Iron:	Blue
Acid mucin:	Blue
Cellular nuclei:	Red

WARNING: reagents are stored at +4°C/+8°C, we recommend to keep them at room temperature before use for at least 10 minutes.
If they are used cold, the reaction speed is significantly reduced.

Preparation	Paraffin section
Control	Cartilage
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	30 min
Suggested fixatives	Formalin
Critical step	Use positive control. Do not use metallic objects. Use fresh reagents.



Intestine. Blue stain for mucins and black for iron deposits.

HISTOLOGICAL STAINING KIT

LONG GIEMSA acc. LENNERT
code 010225

IVD CE

Description
Polychromatic stain for an optimal morphological visualization of haemolymphopoietic tissue. It is intended for use in histological visualization of blood parasites, embedding bodies, mast cells and *Helicobacter Pylori*.

Staining protocol
Where necessary, drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

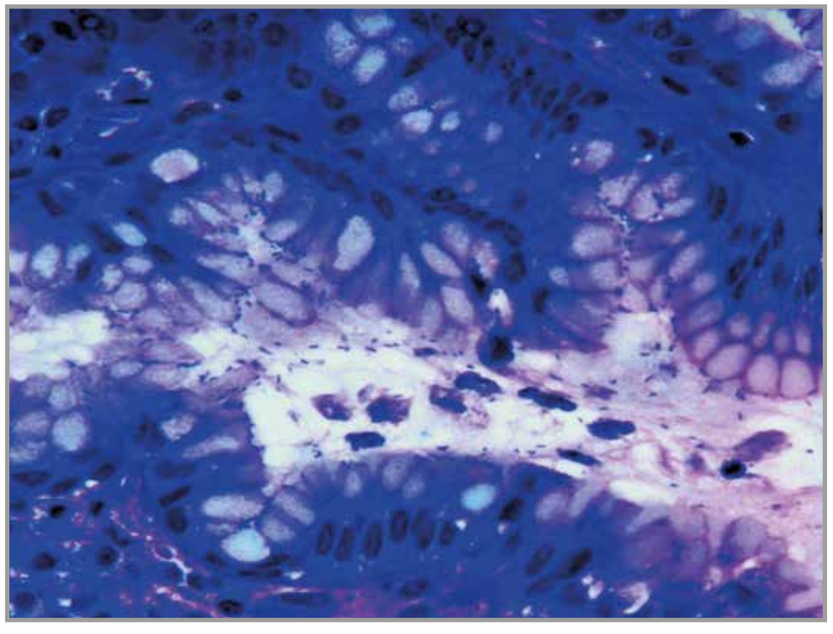
1. Giemsa solution: 7 ml of **reagent A (Giemsa)** diluted in 60 ml of distilled water
2. Deparaffinize and hydrate section to distilled water
3. Immerse slides in Giemsa working solution (see step 1) for 60 minutes
4. Wash in distilled water
5. **Reagent B (Differentiation solution)** for 10 seconds
6. Wash in distilled water
7. Immerse slides in **reagent C (Alcoholic solution)** for 5 minutes
8. **Reagent D (Isowave)** for 2 minutes
9. **Reagent E (Isowave)** for 2 minutes
10. Clear and mount with balsam

WARNING: **reagent C** can be used again after filtration. **Reagents D** and **E** can be used again without filtration.

Results

Nuclei:	Blue
Bacteria and protozoan:	Dark Blue
Background:	from Pink to light Blue

Preparation	Paraffin section
Control	Stomach (reported case of *Helicobacter Pylori*)
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	1h 10 min
Suggested fixatives	Formalin
Critical step	None



Stomach, positive case for *Helicobacter Pylori*.

HISTOLOGICAL STAINING KIT

LUXOL FAST BLUE acc. KLUWER-BARRERA
code 010226

IVD CE

Description
The Kit is designed for demonstrating myelin and Nissl substance in histological sections.

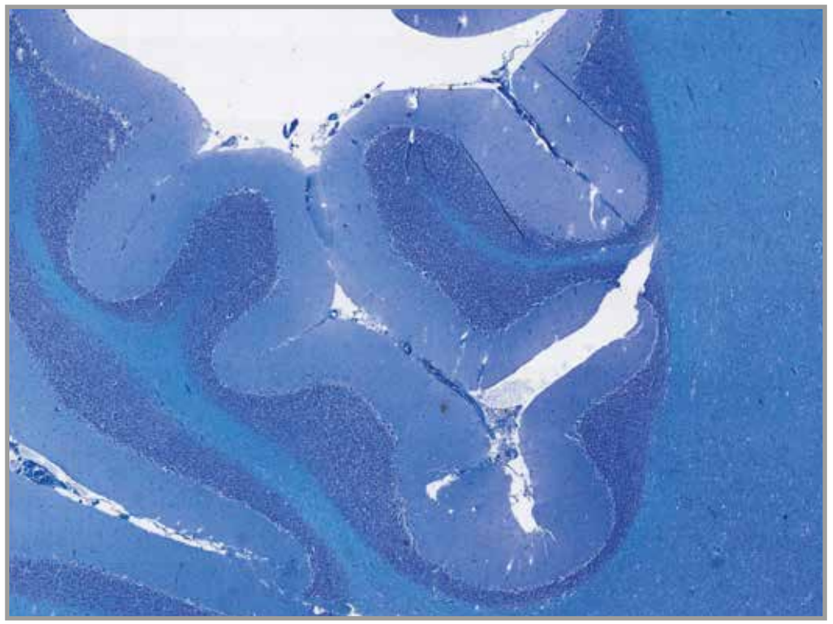
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to ethyl alcohol 95°
2. **Reagent A (Luxol Fast Blue)**, incubate overnight at +37°C (or 2 hours at +60°C)
3. Wash in ethyl alcohol 95° until the complete crystals melting
4. Wash in distilled water
5. **Reagent B (Lithium carbonate)** for 30 seconds (verify at microscope that the gray matter differentiates visually from white one). Incubate again if necessary
6. Immerse section in ethylic alcohol 70° until myelinic fibers turn blue on colorless background (we recommend to verify at microscope)
7. Wash in distilled water (twice)
8. Cover section with 10 drops of **reagent C (Cresyl Violet)** + 5 drops of **reagent D (Acid Activation Buffer)** for 10-20 minutes at +56°C
9. Ethyl alcohol 95° until Nissl substance turns pale Pink
10. Dehydrate in absolute ethyl alcohol
11. Clear and mount with balsam

Results

Myelin:	Blue turquoise
Neurons and glial nuclei:	Pink to Violet
Nissl substance:	Pale pink

Preparation	Paraffin sections
Control	Nervous system
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	24h + 25 min with overnight incubation 2h 30 min with incubation at +60°C
Suggested fixatives	Formalin
Critical step	Reagent temperature



Brain. Cerebral cortex stain.

HISTOLOGICAL STAINING KIT

MALLORY TRICHROME acc. McFARLANE
code 010227

IVD CE

Description
The Kit is intended for use in histological visualization of connective tissue, with particular affinity for collagen, reticulum, cartilage, bones and amyloid.

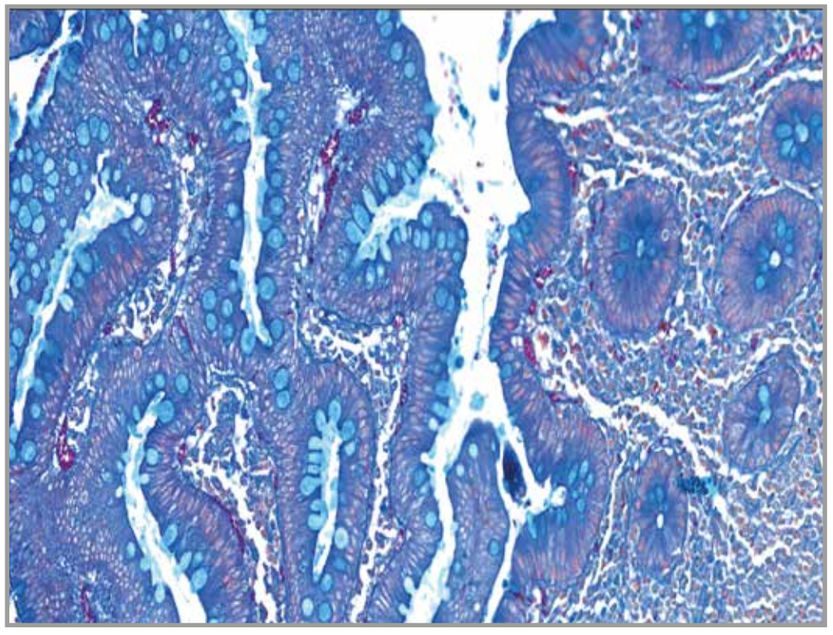
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. **Reagent A (Acid fuchsin)** for 5 minutes
3. Wash quickly in distilled water
4. **Reagent B (Phosphomolybdic Acid)** for 3 minutes
5. Drain reagent and go to the next step
6. **Reagent C (Mallory polychrome solution)** for 5 minutes
7. Differentiate some seconds in ethyl alcohol 95°
8. Dehydrate, clear and mount with balsam

Results

Collagen fibrils:	Dark blue
Cartilage, bone, amyloid:	Varying shade of blue
Nuclei, myofibrils, neuroglia fibrils, axones, fibrin:	Red
Granules of acidophilic hypophysis cells:	Orange
Erythrocytes, myelin and nucleoli:	Yellow
Elastic fibrils:	from pale Pink to Yellow or colorless
Smooth muscle:	Violet

Preparation	Paraffin section
Control	Parathyroid, smooth muscle
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	15 min
Suggested fixatives	Formalin
Critical step	None



Intestine. Mallory stain. Non-pathological tissue.

HISTOLOGICAL STAINING KIT

MASSON FONTANA
code 010228

IVD CE

Description
The Kit supplies reagents for Masson Fontana stain to show melanin in tissue. Melanin is a pigment normally present in the hair, skin, retina and in some areas of the central nervous system and is showed by an argentafin reticular fibers reaction. Some carcinoids have argentaffins granules. The pre-treatment allows to differentiate, in a specific way, myelin from argentaffins substance.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.
Deparaffinize and hydrate section to distilled water.

PRE-TREATMENT (to perform in a control section):
1. 5 drops **reagent B (Permanganate Potassium)** + 5 drops **reagent C (Acid Activation Buffer)** for 30 minutes
2. Wash in distilled water
3. Cover the section with **reagent D (Oxalic Acid)** for 5 minutes

MASSON FONTANA STAINING:
4. **Reagent A (Ammoniacal solution)** overnight at room temperature (alternatively 30-40 minutes at +56°C)
5. Wash more times in distilled water
6. **Reagent E (Sodium Thiosulphate)** for 1-2 minutes
7. Wash more times in distilled water
8. **Reagent F (Kernechtrot)** per 5 minutes
9. Wash in distilled water
10. Dehydrate, clear and mount with balsam

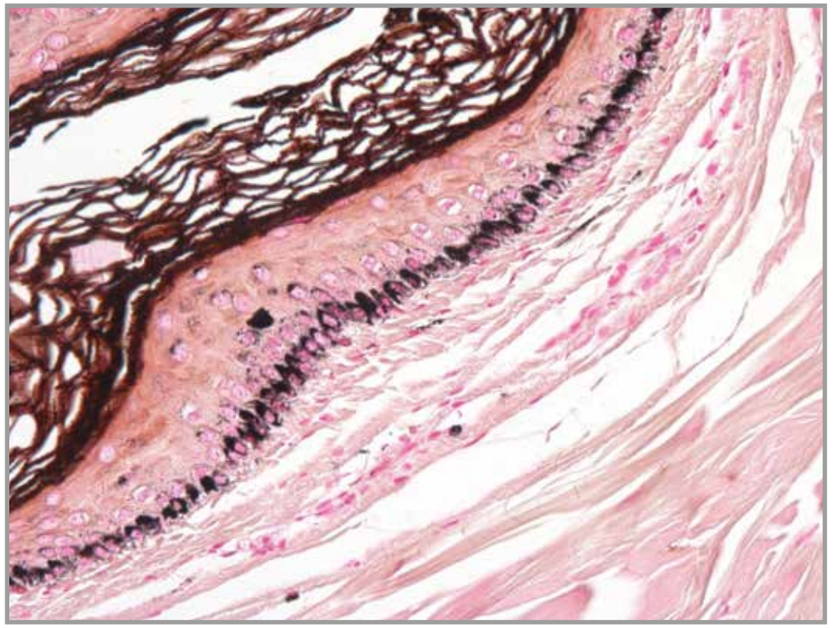
WARNING: staining involves the use of alkaline solutions, which can cause section detachment from the slide. We recommend the use of positively charged slides.

Results

Melanin, argentaffine substances:	Black
Nuclei:	Red
Background:	Pink-Red

WARNING: reagents are stored at +4°C/+8°C, we recommend to keep them at room temperature before use for at least 10 minutes. If they are used cold, the reaction speed is significantly reduced.

Preparation	Paraffin section
Control	Skin, melanin
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	35 min + 24h with overnight incubation 1h 30 min with incubation at +56°C
Suggested fixatives	Formalin
Critical step	Reagent temperature. Pay attention to the possible detachment of the section from the slide



Skin. Black granular stain for melanin.

HISTOLOGICAL STAINING KIT

MASSON TRICHROME acc. CAPELLI (WITH ANILINE BLUE)

code 010210

IVD CE

Description

The Masson trichrome is a special stain to show connective tissue, collagen, reticular and muscle fibers in paraffin sections. In particular this protocol is characterized by a blue stain of collagen and reticulated fibers. If necessary to counterstain with green, see Kit 010224 (Goldner Trichrome).

Staining protocol

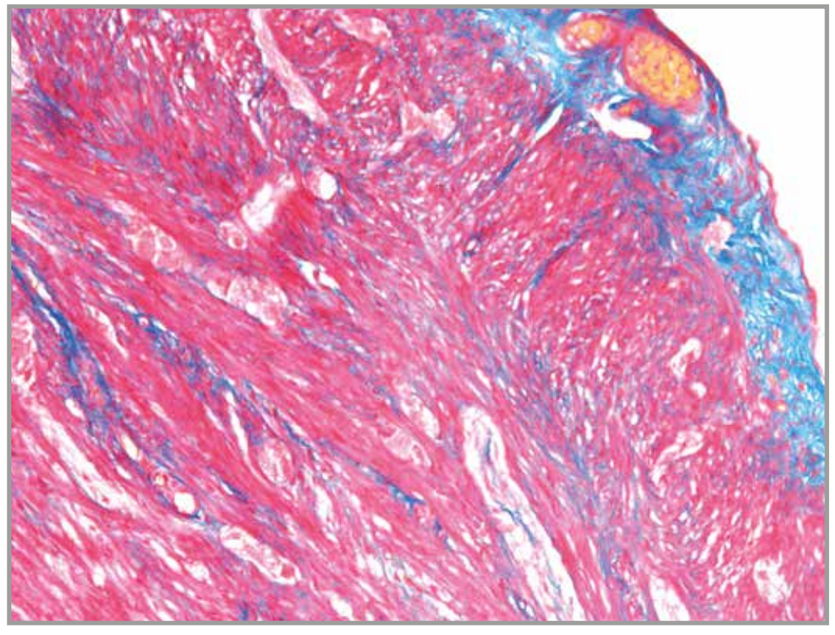
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. Cover the section with Weigert hematoxylin solution for 10 minutes
3. Running tap water for at least 5 minutes
4. **Reagent C** for 4 minutes
5. Wash in distilled water for 30 seconds
6. **Reagent D** for 4 minutes
7. **Reagent E** for 4 minutes
8. **Reagent F** for 5 minutes
9. Dehydrate quickly, clear and mount with balsam

Results

Nuclei:	Brown-Black
Muscle fibers, keratin, cytoplasm:	Bright Red
Collagen, mucus:	Blue
Erythrocytes:	Yellow-Orange

Preparation	Paraffin section
Control	Intestine, liver
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	1h 10 min
Suggested fixatives	Formalin
Critical step	None



Umbilical cord. Masson trichrome for connective tissue.

HISTOLOGICAL STAINING KIT

MAY GRUNWALD GIEMSA acc. ROMANOWSKY FOR TISSUE SECTIONS

code 010229

IVD CE

Description

The Kit is suitable for cell typing of haemolyphopoietic tissue and to visualize parasites. It can be used during endothelial reticulum identification too.

Staining protocol

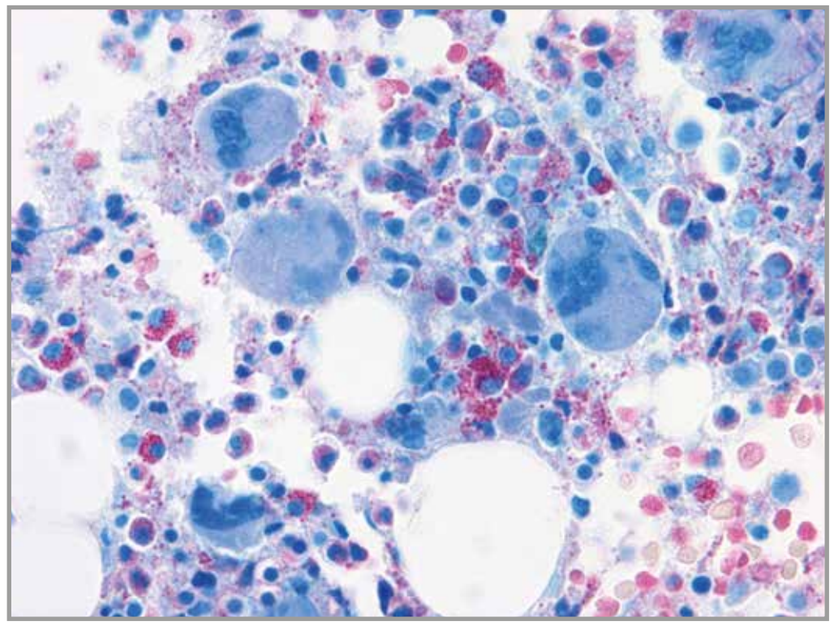
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. Dilute the **reagent A (May Grunwald)** with distilled water with ratio 1:4
3. Cover the section with the solution (STEP 2) for 20 minutes at +37°C
4. Wash in distilled water
5. Dilute 20 drops of **reagent B (Giemsa)** with 10 ml of distilled water
6. Cover the section with the solution (STEP 5) for 40 minutes at +37°C
7. Wash in distilled water
8. Cover the section with **reagent C (Differentiation Buffer)** for 30 seconds
9. Drain the section and dry it with filter paper
10. Dehydrate with solution 1:1 of absolute ethyl alcohol and acetone
11. Clear and mount with balsam

Results

Nuclei:	Violet
Basophile cytoplasm:	from light Blue to dark Blue
Acidophil cytoplasm:	from light Red to Pink

Preparation	Paraffin section
Control	Osteomidollar biopsies
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	1h 5 min
Suggested fixatives	Formalin
Critical step	Reagent dilution



Osteomidollar biopsy. MMG staining.

CYTOLOGICAL STAINING KIT

MAY GRUNWALD GIEMSA FOR SMEARS
code 010802

IVD CE

Description
Main protocol for cell typing and parasite identification on blood smears. This Kit is particularly suitable for the detection of *Trichomonas* in vaginal smears.

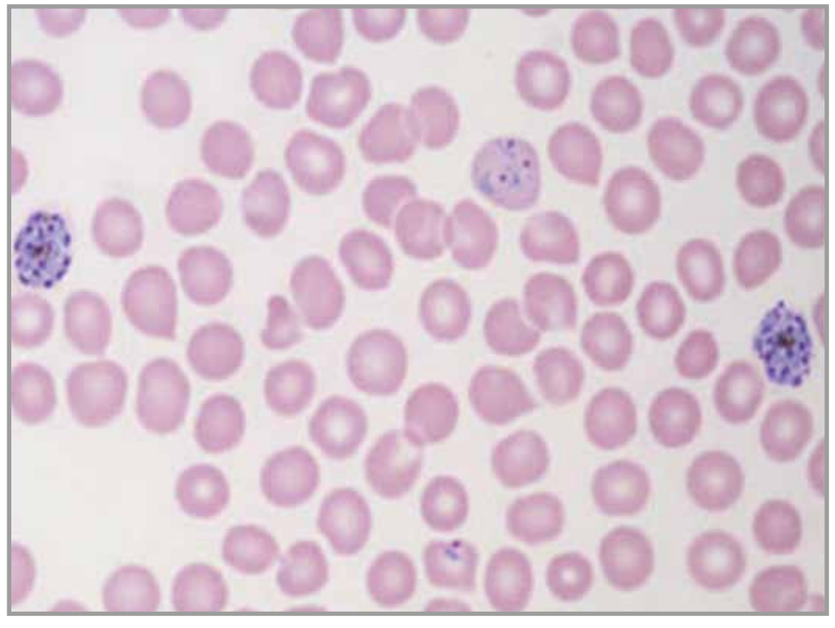
Staining protocol
To avoid section excessive drying, use an incubator box.

1. Dilute **reagent B (Acetate buffer)** with distilled water (1:10). Store the buffer solution at 4°C/+8°C. The solution will be necessary to dilute reagent C
2. Leave the smears to dry in the open air
3. Immerse slides in the **reagent A (May Grunwald)** for 5 minutes
4. Wash in running tap water for 1 minute
5. Prepare Giemsa solution: 10 ml of **reagent C (Giemsa)** + 90 ml of buffer solution (STEP 1). Immerse slides for 15 minutes
6. Wash in running tap water for 1 minute
7. Leave the smears to dry in the open air for 10 minutes (attention: do not use heating sources)
8. Clear and mount with balsam

Results

Nuclei:	Violet
Basophil cytoplasm:	from pale Blue to dark Blue
Acidophil cytoplasm:	from pale Red to Pink

Preparation	Paraffin section
Control	Peripheral blood smear
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	35 min
Suggested fixatives	Non-foreseen
Critical step	Reagent dilution, smear quality (thickness, length)



Blood smear. Stain to highlight the different cell components.

HISTOLOGICAL STAINING KIT

MOVAT PENTACHROME STAIN
code 010247

IVD CE

Description
The Kit is intended for use in histological visualization of: collagen, muscle tissue, reticular fibers, mucins and fibrin in paraffin sections.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

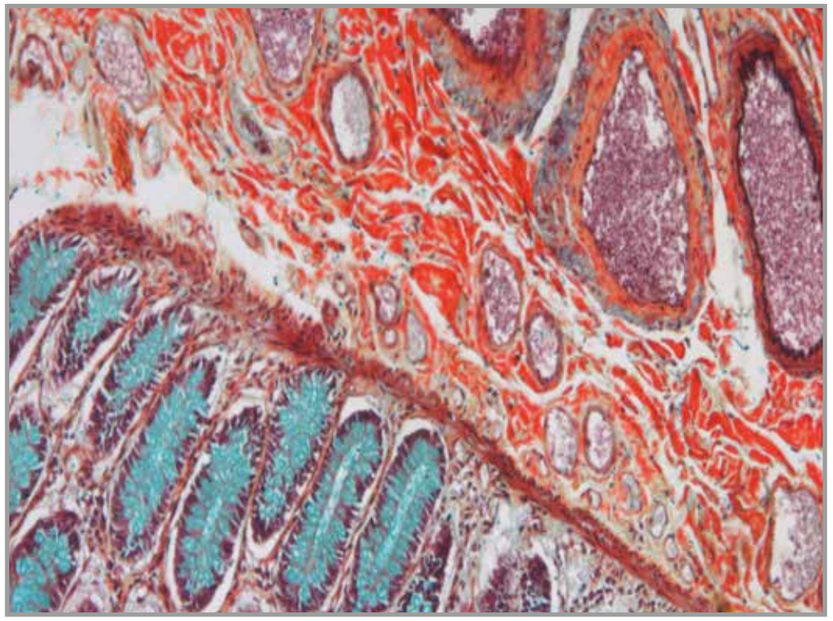
1. Deparaffinize and hydrate to distilled water
2. **Reagent A (Alcian Blue)** for 20 minutes
3. Wash in running tap water for 5 minutes
4. **Reagent B (Alkaline alcoholic solution)** for 60 minutes
5. Wash in running tap water for 10 minutes
6. Prepare **reagent C** adding reagents C1 and C2: **C1 (Hematoxylin 1 solution)** + **C2 (Hematoxylin 2 solution)** *
7. Cover the section with the solution for 15 minutes
8. Wash in distilled water
9. **Reagent D (Ferric Chloride)** until elastic fibers turn black
10. Wash in distilled water
11. Wash in 0.5% acetic acid solution in distilled water
12. **Reagent E (Tiosulphate Sodium)** for 1 minute
13. Wash in running tap water for 10 minutes
14. Wash in distilled water
15. **Reagent F (Briebrich Scarlet solution – Acid Fuchsin)** for 3 minutes
16. Wash in 0.5% acetic acid solution in distilled water
17. **Reagent G (Phosphotungstic Acid)** for 10 minutes
18. Wash in 0.5% acetic acid solution in distilled water
19. Immerse slides in absolute ethyl alcohol (2 quick washings)
20. **Reagent H (Alcoholic Safranin)** for 15 minutes
21. Wash in absolute ethyl alcohol for 2 minutes
22. Clear and mount with balsam

*The hematoxylin solution (**reagent C1**+**reagent C2**) is stable for about 6-9 months; store at room temperature. Alternatively, add 2 drops of **reagent C1** + 1 drop **reagent C2** and cover the section.

Results

Nuclei and elastic fibers:	Brown-Black
Collagen and reticular fibers:	Yellow
Mucins:	Blue
Fibrinoid substance, fibrin:	Dark Red
Muscle:	Red

Preparation	Paraffin section
Control	Intestine, liver
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	2h 30 min
Suggested fixatives	Formalin
Critical step	Reagent C with limited stability



Intestine, colon. Polychromic stain. Non-pathological tissue.

HISTOLOGICAL STAINING KIT

MUCICARMINE acc. MAYER
code 010245

IVD CE

Description
The Kit supplies reagents to visualize acid mucins with mucicarmine stain. Counterstaining with Weigert hematoxylin and Yellow Methanil.

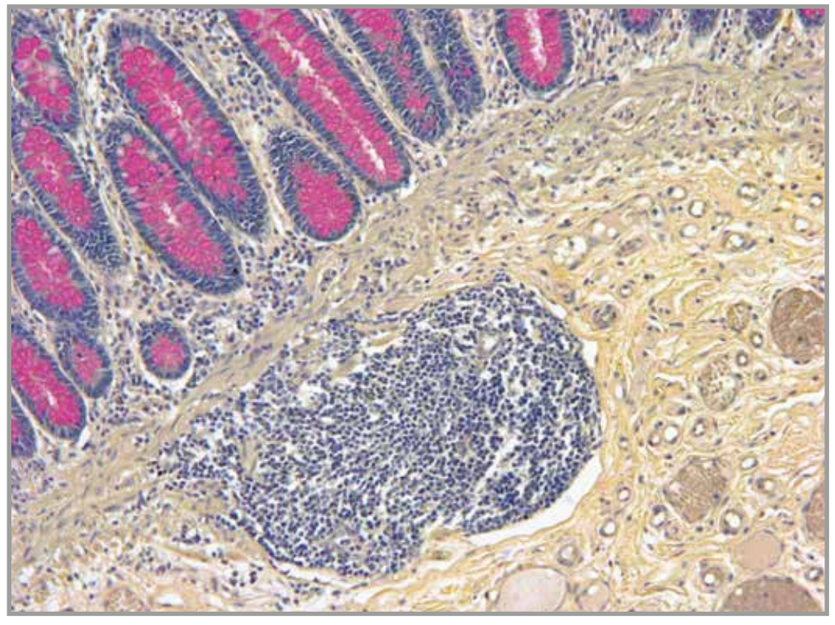
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. 5 drops of **reagent A (Weigert hematoxylin sol. A)** + 5 drops of **reagent B (Weigert hematoxylin sol. B)** for 2-5 minutes
3. Wash in running tap water for 10 minutes
4. Prepare the mucicarmine solution: 10 drops of **reagent C (Mayer Mucicarmine)** in 1 ml of distilled water
5. Cover the section with the solution for 60 minutes at room temperature
6. Wash in distilled water
7. **Reagent D (Yellow Methanil)** for 1 minute
8. Wash in distilled water
9. Dehydrate quickly, clear and mount with balsam

Results

Nuclei:	Black
Acid mucins:	different shades of Red
Other components, neutral mucins:	Light Yellow

Preparation	Paraffin section
Control	Intestine
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	1h 25 min
Suggested fixatives	Formalin
Critical step	Use fresh sections; dilute reagent C before the use



Intestine. Pink stain of mucins.

HISTOLOGICAL STAINING KIT

OIL RED O acc. JOHNSON
code 010303

IVD CE

Description
The Kit supplies reagents to show lipids with Oil Red O stain. Oil Red O is a stain with lisochromic features. Due to these features, the chromophore is soluble in lipids that turn its color.

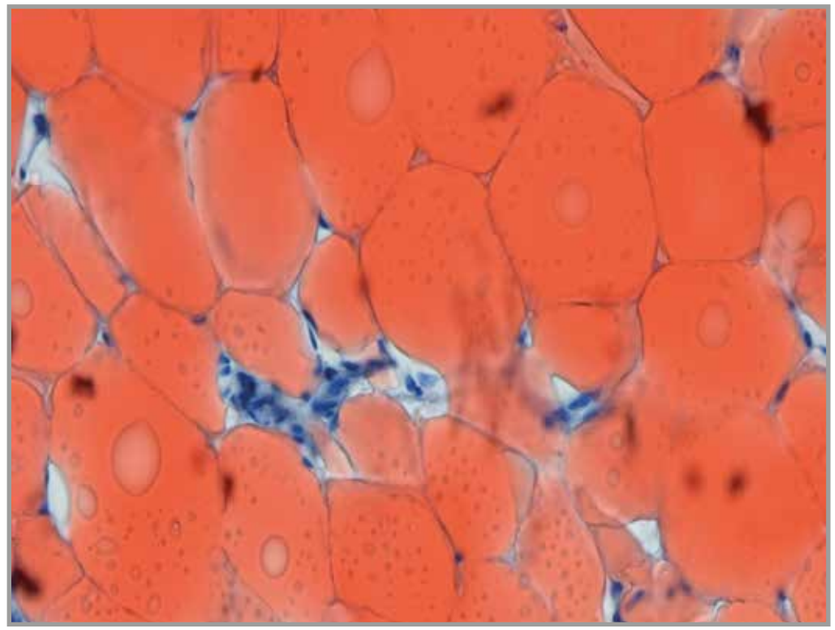
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Hydrate the section to distilled water
2. Working solution: 8 ml of **reagent A (Oil Red O)** + 5 ml of **reagent B (Activation basic buffer)**. Let stand at least 10 minutes before the use*
3. Cut section at cryostat
4. Fix the section immersing the slide in ready to use formalin (non-equipped reagent in the Kit) for 1 minute
5. Wash in running tap water
6. Cover the section with the working solution (STEP 1) for 10 minutes
7. Wash in running tap water
8. **Reagent C (Mayer hematoxylin)** for 3 minutes
9. Wash in running tap water for 1-2 minutes
10. Mount with watery mounting media

* The diluted solution is stable up to 24 hours

Results
Lipids: Red
Nuclei: Blue-Violet

Preparation	Section at cryostat
Control	Fat tissue
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	25 min
Suggested fixatives	Formalin
Critical step	Let stand the working solution for 10 minutes before the use



Frozen tissue. Lipid Red stain.

HISTOLOGICAL STAINING KIT

PARALDEHYDE FUCHSIN acc. GOMORI
code 010235

IVD CE

Description
The Kit is intended for use in histological visualization of elastic fibers and pancreas endocrine components with visualization of nuclei in blue and the connective tissue in green.

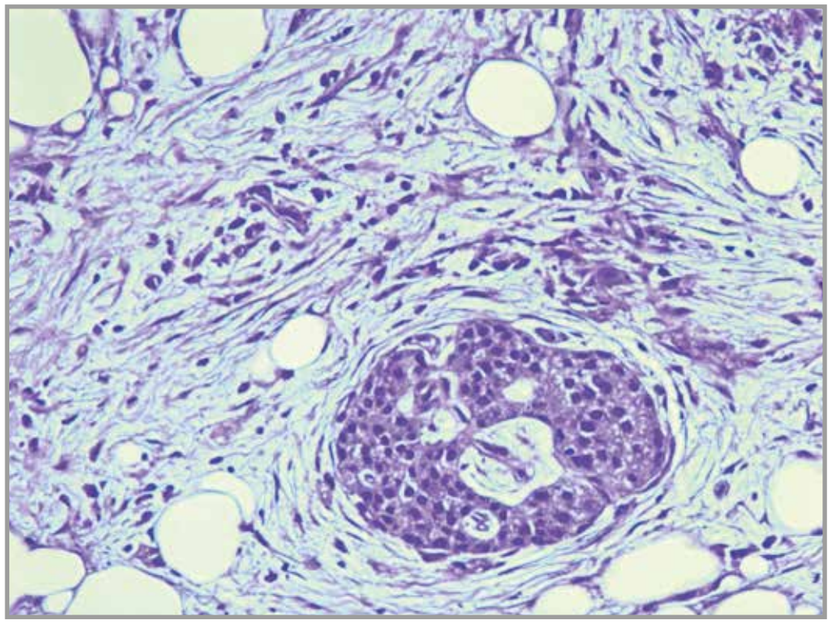
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. **Reagent A (Potassium Permanganate)** 5 drops + **reagent B (Activation acid buffer)** 5 drops for 10 minutes
3. Wash in distilled water
4. **Reagent C (Oxalic Acid)** for 5 minutes
5. Wash in distilled water
6. **Reagent D (Differentiation solution)** for 5 minutes. Drain slide and go to the next step
7. **Reagent E (Paraldehyde Fuchsin)** for 20 minutes. Drain slide and go to the next step
8. **Reagent F (Differentiation solution)** for 5 minutes
9. Wash in distilled water
10. **Reagent G (Mayer Hematoxylin)** for 5 minutes
11. Wash in running tap water for 5 minutes
12. **Reagent H (Light Green)** for 5 minutes
13. Wash in distilled water
14. Dehydrate quickly, clear and mount with balsam

Results

Pancreas beta cells granules, elastic fibers, sulphated mucins, mast cells:	Dark Violet
Nuclei:	Blue-Violet
Connective tissue:	Green

Preparation	Paraffin section
Control	Pancreas
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	1h
Suggested fixatives	Formalin
Critical step	None



Pancreas. β cells granules in dark violet.

HISTOLOGICAL STAINING KIT

P.A.S. - PERIODIC ACID SCHIFF acc. HOTCHKISS-McMANUS
code 010231

IVD CE

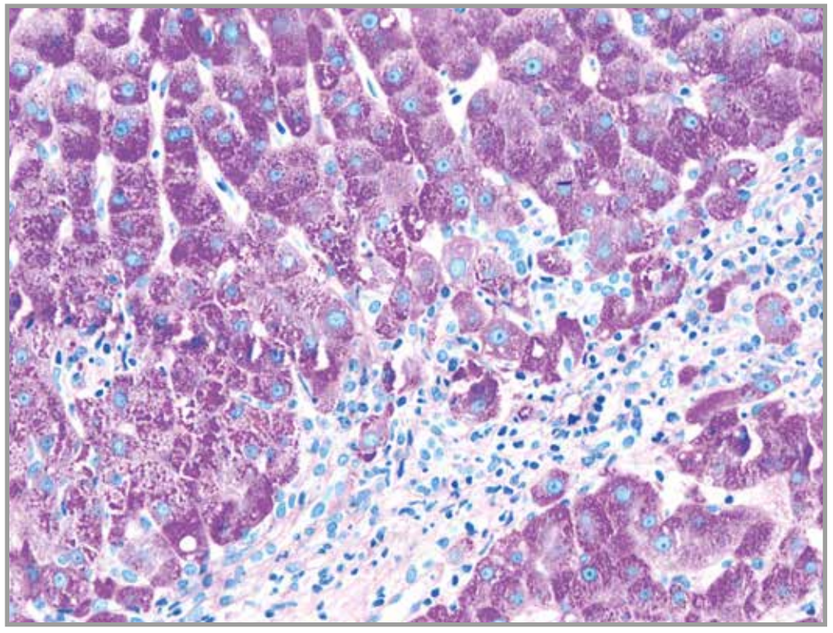
Description
The Kit supplies reagents for P.A.S. (Periodic Acid Schiff) staining. The Schiff reagent is a watery, colorless solution, used in combination with periodic acid to highlight aldehyde groups. The P.A.S. stain is mainly used to visualize the presence of glycogen but also of glycoproteins. Many tissues may result P.A.S. positive, for example: the ground substance of connective tissues, cartilage, bones, mucous cells and glands, kidney structures, hypophysis basophil cells, thyroid colloid substance.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Periodic acid solution: melt **reagent A (Periodic Acid)** in 50 ml of distilled water. Stir until the complete powder melting. Store the solution at +4°C/+8°C in tightly closed container. It has validity of 12 months
2. Deparaffinize and hydrate to distilled water
3. Cover the section with periodic acid solution (STEP 1) for 20-30 minutes
4. Wash in distilled water
5. **Reagent B (Schiff reagent)** for 10-30 minutes (until the section turns magenta)
6. Wash in distilled water
7. Washing solution: 80 ml of distilled water + 10 drops of **reagent C (Metabisulphite Potassium)** + 10 drops of **reagent D (Hydrochloridric Acid)**
8. Immerse slides in washing solution (STEP 7) for 10 minutes
9. Wash in distilled water
10. **Reagent E (Mayer hematoxylin)** for 2 minutes
11. Wash in running tap water for 5 minutes
12. Dehydrate quickly, clear and mount with balsam

Results
Nuclei: Blue-Violet
Positive P.A.S. substances (glycogen): Magenta

Preparation	Paraffin section – cytological specimen
Control	Liver
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	1h 20 min for paraffin sections 30-40 min for cytological specimens
Suggested fixatives	Formalin
Critical step	None



Liver. Glycogen shown in magenta. Nuclei counterstaining in blue.

HISTOLOGICAL STAINING KIT

P.A.S. - PERIODIC ACID SCHIFF acc. MOREL-MARONGER
code 010232

IVD CE

Description
The Kit supplies reagents for P.A.S. (Periodic Acid Schiff) staining. The Schiff reagent is a watery, colorless solution, used in combination with periodic acid to visualize aldehyde groups. The P.A.S. stain is mainly used to visualize the presence of glycogen but also of glycoproteins. Many tissues may result P.A.S. positive, for example: the ground substance of connective tissues, cartilage, bones, mucous cells and glands, kidney structures, hypophysis basophil cells, thyroid colloid substance.
The staining protocol acc. Morel-Maronger is characterized by staining of connective tissue with Picroindingo Carmine.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

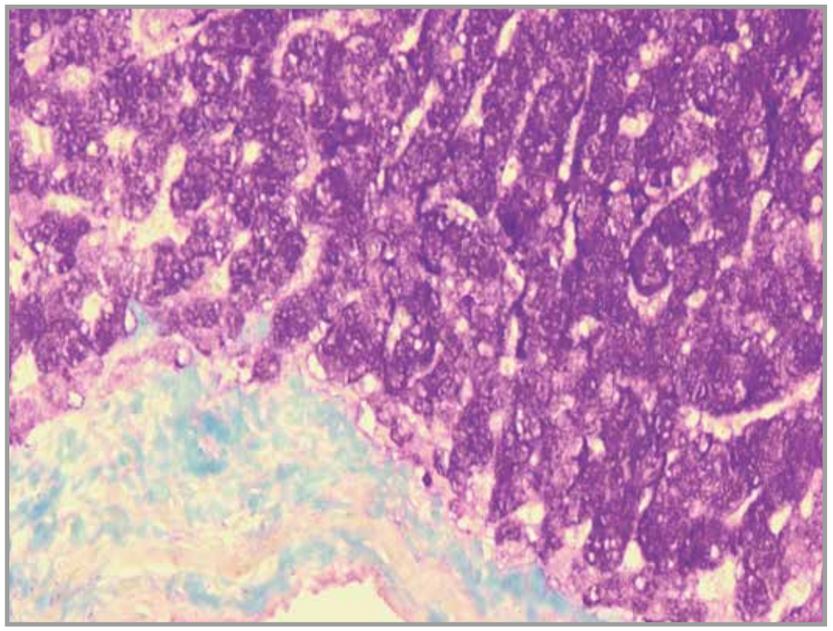
1. Periodic acid solution: melt **reagent A (Periodic Acid)** in 50 ml of distilled water. Stir until the complete powder melting. Store the solution at +4°C/+8°C in tightly close container. It has validity of 12 months
2. Deparaffinize and hydrate to distilled water
3. Cover the section with periodic acid solution (STEP 1) for 20-30 minutes
4. Wash in distilled water
5. Cover the section with **reagent B (Schiff reagent)** for 15-30 minutes
6. Wash in distilled water
7. Prepare solution: 80 ml of distilled water + 10 drops of **reagent C (Metabisulphite Potassium)** + 10 drops of **reagent D (Hydrochloridric Acid)**
8. Immerse slides in washing solution (STEP 7) for 5 minutes
9. Wash in distilled water
10. Cover the section with **reagent E (Picroindingo Carmine)** for 5 minutes
11. Wash quickly in distilled water
12. Dehydrate quickly, clear and mount with balsam

Results

Positive P.A.S. substances (glycogen):	Magenta
Connective tissue, muscle, neuroglia, erythrocytes:	Yellow-Green

WARNING: reagents are stored at +4°C/+8°C, we recommend to keep them at room temperature for about 10 minutes before the use. If they are used cold, the reaction speed is significantly reduced.

Preparation	Paraffin section
Control	Liver
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	1h 10 min
Suggested fixatives	Formalin
Critical step	Use reagents at room temperature



Liver. Glycogen shown in magenta. Tissue connective counterstaining in yellow-green.

HISTOLOGICAL STAINING KIT

P.A.S. - PERIODIC ACID SCHIFF acc. PEARSE
code 010233

IVD CE

Description
The Kit supplies reagents for P.A.S. (Periodic Acid Schiff) staining. The Schiff reagent is a watery, colorless solution, used in combination with periodic acid to highlight aldehyde groups. The P.A.S. stain is mainly used to visualize the presence of glycogen but also of glycoproteins. Many tissues may result P.A.S. positive, for example: the ground substance of connective tissues, cartilage, bones, mucous cells and glands, kidney structures, hypophysis basophil cells, thyroid colloid substance.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

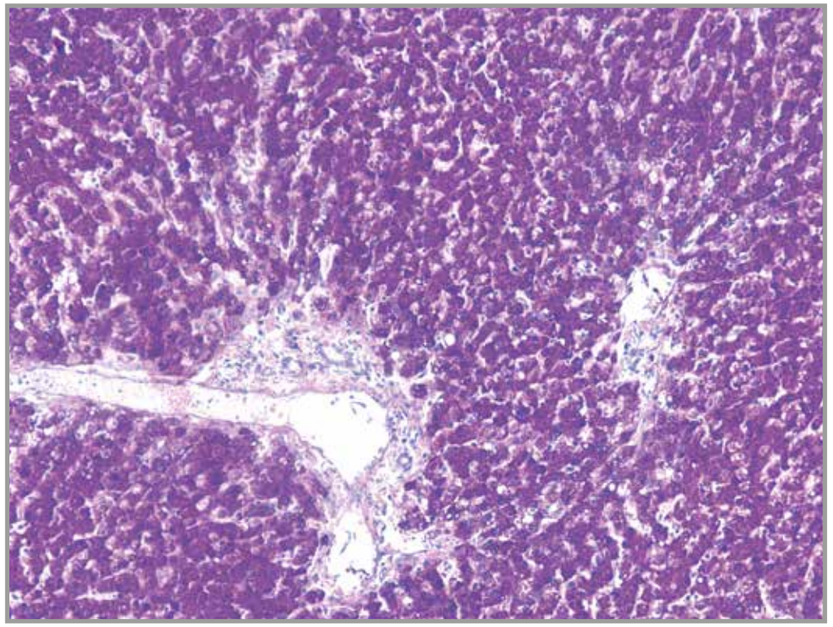
1. Deparaffinize and hydrate to distilled water
2. **Reagent A (Periodic Acid)** for 10 minutes
3. Wash in distilled water
4. **Reagent B (Schiff reagent)** for 30 minutes
5. Wash in distilled water
6. Working solution: 80 ml of distilled water + 10 drops of **reagent C (Metabisulphite Potassium)** + 10 drops of **reagent D (Hydrochloridric Acid)**
7. Immerse slides in washing solution (STEP 6) for 10 minutes
8. Wash in distilled water
9. Cover the section with **Reagent E (Mayer hematoxylin)** for 2 minutes
10. Wash in running tap water for 5 minutes
11. Dehydrate quickly, clear and mount with balsam

Results

Nuclei:	Blue-Violet
Positive P.A.S. substances (glycogen):	Magenta

WARNING: reagents are stored at +4°C/+8°C, we recommend to keep them at room temperature for about 10 minutes before the use. If they are used cold, the reaction speed is significantly reduced.

Preparation	Paraffin section
Control	Liver
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	1h
Suggested fixatives	Formalin
Critical step	Use reagents at room temperature



Liver. Glycogen shown in magenta. Nuclei counterstaining in blue.

HISTOLOGICAL STAINING KIT

P.A.S.M. – SILVER METHENAMINE acc. CALLARD code 010234

IVD CE

Description
The Kit supplies reagents of P.A.S.M. (Periodic Acid Silver Methenamine) staining protocol to highlight the basement membranes of kidney tissue. This staining protocol is usually called Gomori's reaction too.
The P.A.S.M. stain visualizes argyrophilic elements, mucopolysaccharides, mycetes and bacteria. The treatment with periodic acid oxidizes the carbohydrates of basement membrane with aldehyde formation: these groups allow to reduce silver with consequent visualization in black of basement membranes.

Staining protocol (*)
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. Cover the section with **Reagent A** – 10 minutes
3. Wash in distilled water – 5 minutes
4. Cover the section with 10 drops of **reagent B** + 10 drops of **reagent C** + 10 drops of **reagent D**
5. Incubate in oven for 60 minutes at +60°C
6. Verify impregnation tone at microscope. If necessary, incubate again. The section should turn tobacco
7. Leave it cool at room temperature for 5 minutes
8. Wash in distilled water
9. Cover the section with **Reagent E** for 5 minutes
10. Wash in distilled water
11. Cover the section with **Reagent F** for 5 minutes
12. Wash in distilled water
13. Immerse the section in Hematoxylin solution – 2 seconds
14. Running tap water – 2 seconds
15. Eosin – 2 seconds
16. Dehydrate quickly, xylol or substitutes. Mount with balsam

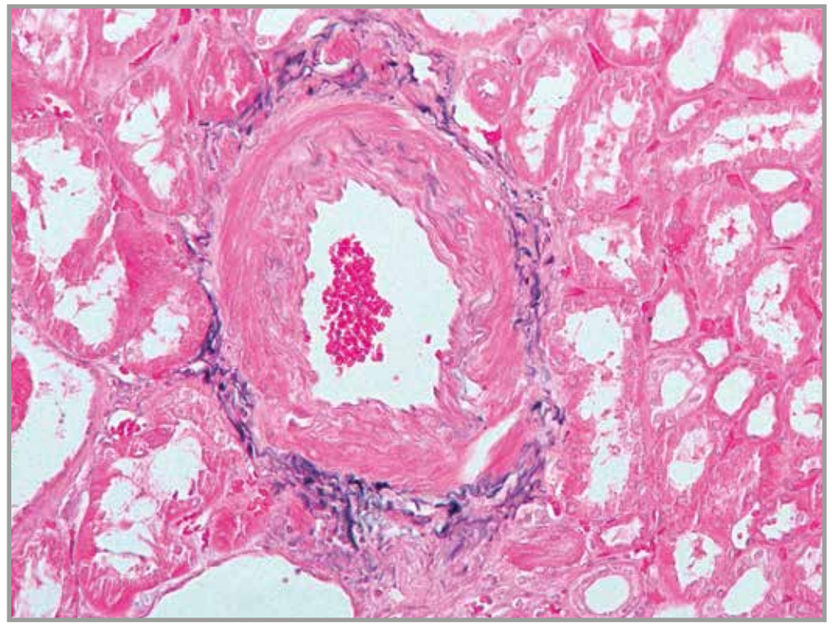
(*) Contrast suggested with hematoxylin and eosin following these steps:
1. Wash in distilled water
2. Immerse in hematoxylin – 2 seconds
3. Running tap water – 2 seconds
4. Eosin – 2 seconds
5. Dehydrate quickly, xylol or substitute. Mount with balsam

WARNING: use excellent distilled water for washings and not use metallic objects. Verify the real oven temperature during incubation step: +60°C are mandatory for the reaction process.

Results
Basement membranes, glycogen, mycetes and bacteria capsule: Black

WARNING: reagents are stored at +4°C/+8°C. We recommend to keep them at room temperature for about 10 minutes before the use. If they are used cold, the reaction speed is significantly reduced.

Preparation	Paraffin section
Control	Kidney
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	1h 45 min
Suggested fixatives	Formalin
Critical step	Reagent temperature. Do not use metallic objects



Kidney. Basement membrane in black.

HISTOLOGICAL STAINING KIT

P.T.A.H. PHOSPHOTUNGSTIC ACID HEMATOXYLIN acc. MALLORY

IVD CE

code 010239

Description
The Kit supplies reagents for staining with phosphotungstic acid hematoxylin used to highlight smooth muscular tissue and central nervous system (CNS) parts.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

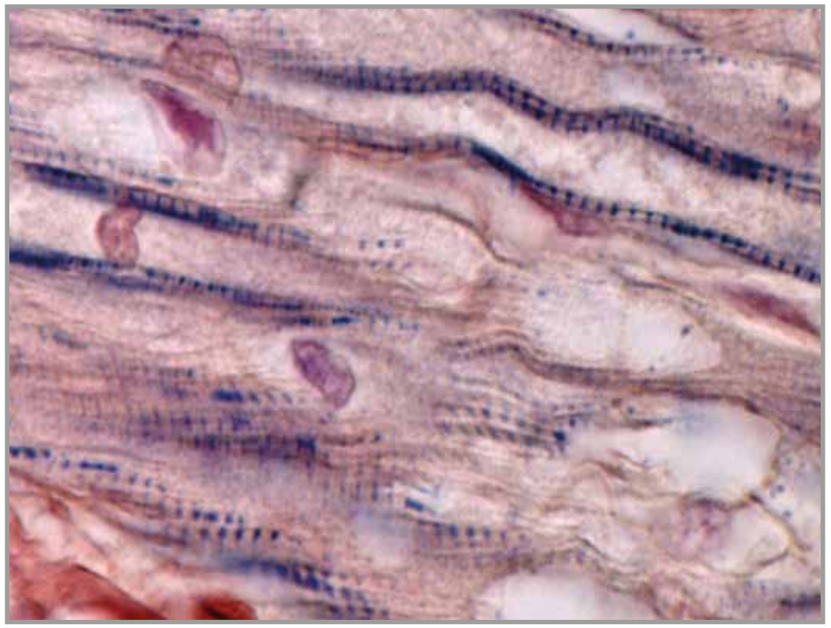
1. Deparaffinize and hydrate to distilled water
2. 5 drops of **reagent A (Potassium Permanganate)** + 5 drops of **reagent B (Activation Acid Buffer)** for 5 minutes
3. Wash in distilled water
4. **Reagent C (Oxalic Acid)** for 5 minutes
5. Distilled water
6. **Reagent D (P.T.A.H.)*** with overnight incubation at room temperature
7. Wash in distilled water for 3-4 seconds
8. Differentiate quickly, clear and mount with balsam

* The **reagent D** can be used again after filtration

Results

Keratin, erythrocytes, nuclei, fibrin, myofibrils, bile canaliculi, neuroglia, elastic fibers, pancreas cells, myelinic fibers:	Dark Blue
Collagen, reticular fibers, mucins e fibrinoid:	Different shades of brick Red
Elastic collagen:	Yellow

Preparation	Paraffin section
Control	Muscular and nervous tissue
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	24h + 15 min (overnight incubation)
Suggested fixatives	Formalin
Critical step	Do not dry slides during overnight incubation



Myocardium. Striated muscular tissue stain.

HISTOLOGICAL STAINING KIT

PERLS
code 010236

IVD CE

Description
The Kit supplies reagents of Perls stain to highlight reactive ferric iron in histological sections. In acid environment, the acid potassium ferrocyanide solution reacts in presence of reactive ferric iron forming an insoluble blue precipitate (Prussian Blue). The Kit provides nuclear red counterstaining.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. Prepare acid potassium ferrocyanide solution: melt n. 1 vial of **reagent A (Potassium Ferrocyanide)** in 80 ml of bidistilled water. Stir until to the complete powder melting then add 20 ml of **reagent B (Hydrochloric Acid)**. Attention: use only well clean glassware; avoid contact with metallic objects
3. Immerse the slides in the solution for 10-30 minutes *
4. Wash in distilled water
5. **Reagent C (Kernechtrot)** for 5 minutes
6. Wash in distilled water
7. Dehydrate quickly, clear and mount with balsam

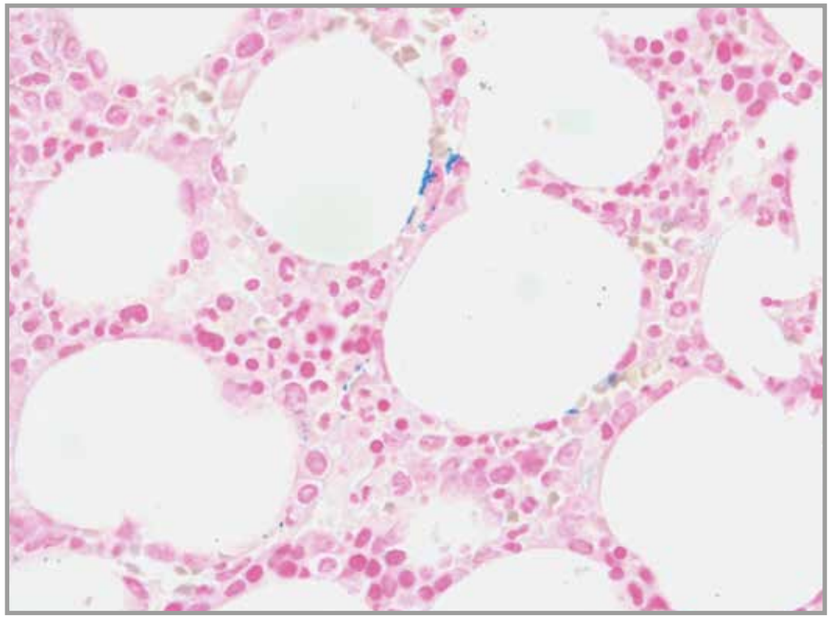
* According to iron present in the tissue, the incubation time may change.

WARNING: this Kit allows to prepare 10 histological jars with the acid potassium ferrocyanide solution (100 ml) that should be prepared soon before the use. Use it again could bring to false positives.

Results
Reactive ferric iron: Blue
Cell nuclei: Red

It is recommended to use always a positive control tissue

Preparation	Paraffin section
Control	Hemorrhagic tissue, spleen
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	35 min
Suggested fixatives	Formalin
Critical step	Use fresh reagents



Marrow biopsy. Iron deposits in blue. Red counterstaining.

HISTOLOGICAL STAINING KIT

PERLS-VAN GIESON
code 010237

IVD CE

Description
The Kit supplies reagents of Perls stain to visualize reactive ferric iron in histological sections. The acid potassium ferrocyanide solution reacts in presence of reactive ferric iron forming an insoluble blue precipitate (Prussian Blue).
The Kit supplies as counterstaining the picrofuchsin acc. Van Gieson, a solution obtained by mixing acid fuchsin with picric acid. This solution selectively stains connective tissue, collagen, muscle tissue and thickened epithelium besides glial fibrils and cytoplasm.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. Prepare acid potassium ferrocyanide solution: melt n. 1 vial of **reagent A (Potassium Ferrocyanide)** in 80 ml of bidistilled water. Stir until the complete powder melting then add 20 ml of **reagent B (Hydrochloric Acid)**.
   Attention: use only well clean glassware, avoid contact with metallic objects
3. Immerse the slides in the solution for 10-30 minutes*
4. Wash in distilled water
5. Cover the section with **Reagent C (Picrofuchsin)** for 5 minutes
6. Wash in distilled water
7. Dehydrate quickly, clear and mount with balsam

* According to iron present in the tissue, the incubation time may change

WARNING: this Kit allows to prepare 10 histological jars with the acid potassium ferrocyanide solution (100 ml) that should be prepared soon before the use. Use it again could bring to false positives.

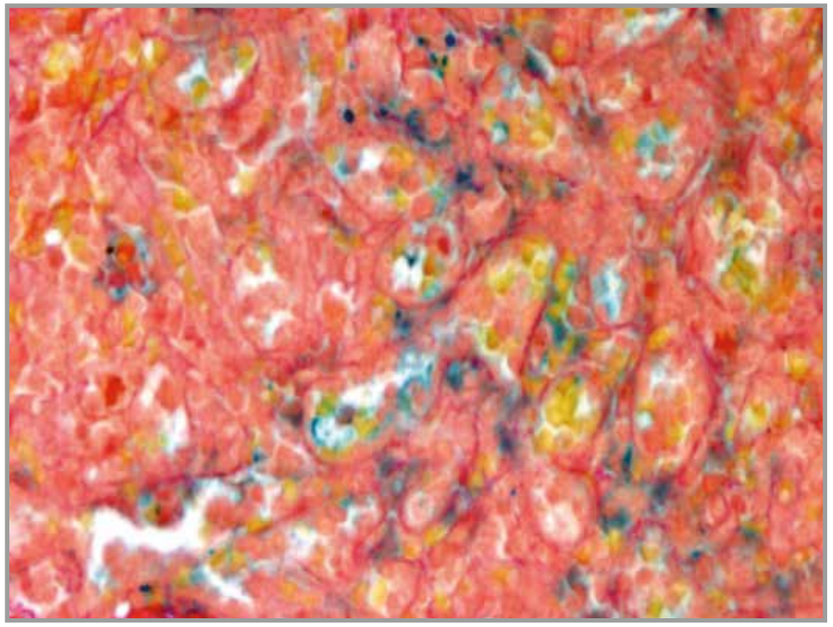
Results

Reactive ferric iron:	Blue
Collagen:	Purple Red
Cytoplasm, muscle, corneum stratum of the epithelium, neuroglia fibers and erythrocytes:	Yellow

It is recommended to use always a positive control tissue

WARNING: reagents are stored at +4°C/+8°C, we recommend to keep them at room temperature for about 10 minutes before the use. If they are used cold, the reaction speed is significantly reduced.

Preparation	Paraffin section
Control	Hemorrhagic tissue, spleen
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	35 min
Suggested fixatives	Formalin
Critical step	Use fresh reagents



Spleen, autopsy case. Iron deposits in blue. Counterstaining of connective tissue with Van Gieson stain.

HISTOLOGICAL STAINING KIT

PICRO MALLORY TRICHROME acc. LENDRUM
code 010238

IVD CE

Description
The Kit is designed for demonstrating the different components of connective tissue.

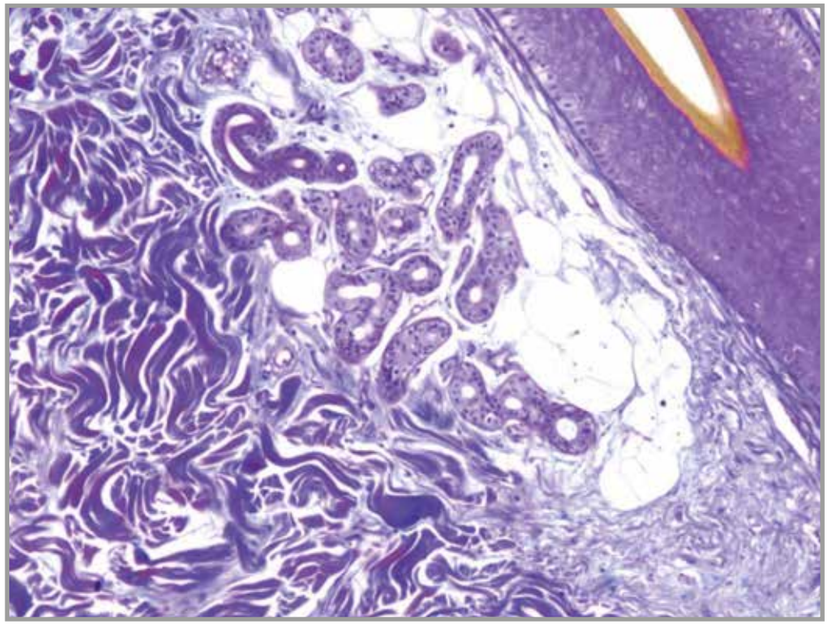
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. Cover the section with 5 drops of **reagent A (Weigert hematoxylin sol. A)** and 5 drops of **reagent B (Weigert hematoxylin sol. B)** for 10 minutes
3. Wash in running tap water for 10 minutes
4. **Reagent C (Picro Mallory Orange G)** for 2 minutes
5. Wash in distilled water
6. **Reagent D (Fuchsin Ponceau)** for 1 minute
7. Wash in distilled water
8. **Reagent E (Phosphomolybdic Acid)** for 15 minutes
9. Wash in distilled water
10. **Reagent F (Aniline blue)** for 1 minute
11. Differentiate quickly in ethyl alcohol 95°
12. Complete dehydration with absolute ethyl alcohol
13. Clear and mount with balsam

Results

Nuclei:	Dark Brown
Collagen fibers:	Dark Blue
Ground substance of cartilage and bone, mucus, basophil granules of hypophysis and amyloid:	different shades of Blue
Neuroglia and fibroglia:	Red
Acidophil granules of hypophysis:	Orange
Myelin and erythrocytes:	Yellow
Elastic fibers:	from pale Pink to Yellow or colorless

Preparation	Paraffin sections
Control	Connective tissue
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	45 min
Suggested fixatives	Formalin
Critical step	None



Skin. Hair follicle stain.

HISTOLOGICAL STAINING KIT

RHODAMINE
code 010248

IVD CE

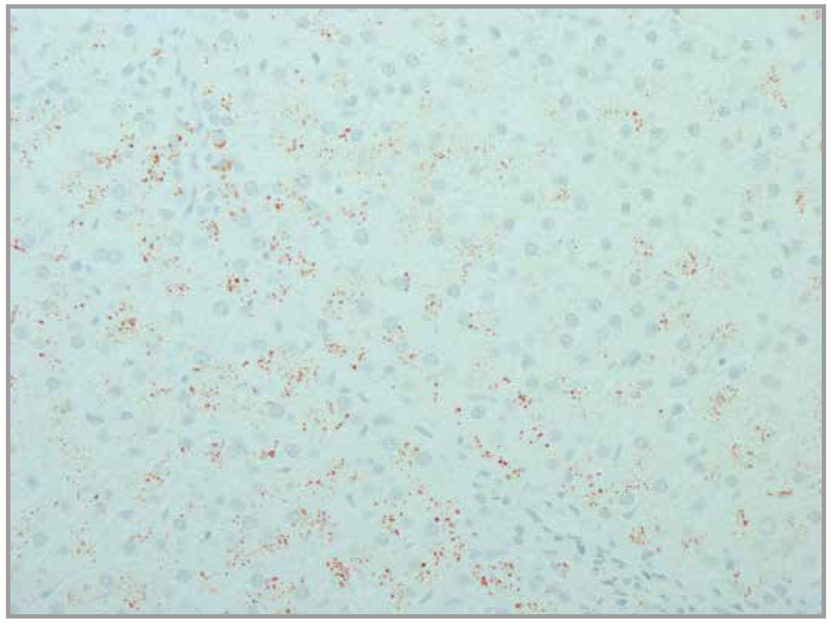
Description
The Kit is designed for demonstrating copper by using rhodamine in hepatic tissue sections.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. Prepare washing buffer solution: 40 ml of distilled water + 10 drops of **reagent A (Buffer Solution 1)** + 10 drops of **reagent B (Buffer Solution 2)**
3. Prepare rhodamine solution: 20 drops of **reagent C (Rhodamine Alcoholic Solution)** in 40 ml of distilled water
4. Immerse slides in rhodamine solution (STEP 3) for 20 hours at +37°C
5. Wash section in buffer solution (STEP 2)
6. **Reagent D (Mayer Hematoxylin)** for 5 minutes
7. Wash section in buffer solution (STEP 2): 3 washings of 1 minute each
8. Dehydrate quickly, clear and mount with balsam

Results
Copper: Brown-Red
Nuclei: Blue-Violet

Preparation	Paraffin section
Control	Tissue with copper deposits (positive case)
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	20h
Suggested fixatives	Formalin
Critical step	Reagent temperature



Fetal liver. Blue – violet staining of nuclei.

HISTOLOGICAL STAINING KIT

SILVER IMPREGNATION
code 010211

IVD CE

Description
The Kit is intended for use in histological visualization of connective tissue fibers. The argirophilic feature of reticular fibers is due to the capacity to bind silver salts, which reduced to metallic silver, give the typical black color.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. Prepare the working solution: 5 drops of **reagent A (Potassium Permanganate)** + 5 drops of **reagent B (Activation Acid Buffer)**. Cover the section with the solution for 5 minutes
3. Wash in distilled water
4. **Reagent C (Oxalic Acid)** for 3 minutes. Wash in distilled water
5. **Reagent D (Sulphate Ammonium Iron)** for 2 minutes. Wash in distilled water
6. **Reagent E (Ammoniacal Solution)** for 2 minutes. Wash in distilled water
7. **Reagent F (Aldehyde Formica in Solution)** for 2 minutes. Wash in distilled water
8. **Reagent G (Sodium Thiosulfate)** for 4 minutes. Wash in running tap water for 5 minutes
9. Dehydrate quickly, clear and mount with balsam

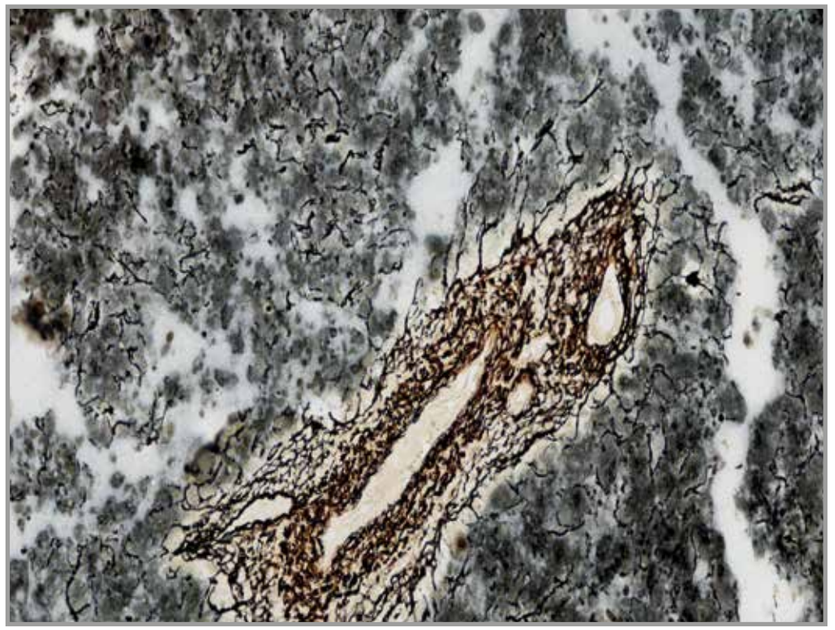
WARNING: the staining involves the use of alkaline solutions which can cause the detachment of the section from the slide.
We suggest the use of positively charged slides.

Results

Reticular and nervous fibers:	Black
Connective tissue:	Brown
Collagen:	Yellow

WARNING: reagents are stored at +4°C/+8°C, we recommend to keep them at room temperature before use for at least 10 minutes.
If they are used cold, the reaction speed is significantly reduced.

Preparation	Paraffin section
Control	Liver
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	25 min
Suggested fixatives	Formalin
Critical step	Do not use metallic objects. Use fresh reagents. Pay attention to the possible detachment of the section from the slide.



Fetal liver. Brown-black pattern of vessels.
Note: the liver reticular structure isn't fully formed as in adult tissue.

HISTOLOGICAL STAINING KIT

SIRIUS RED FOR AMYLOID
code 010306

IVD CE

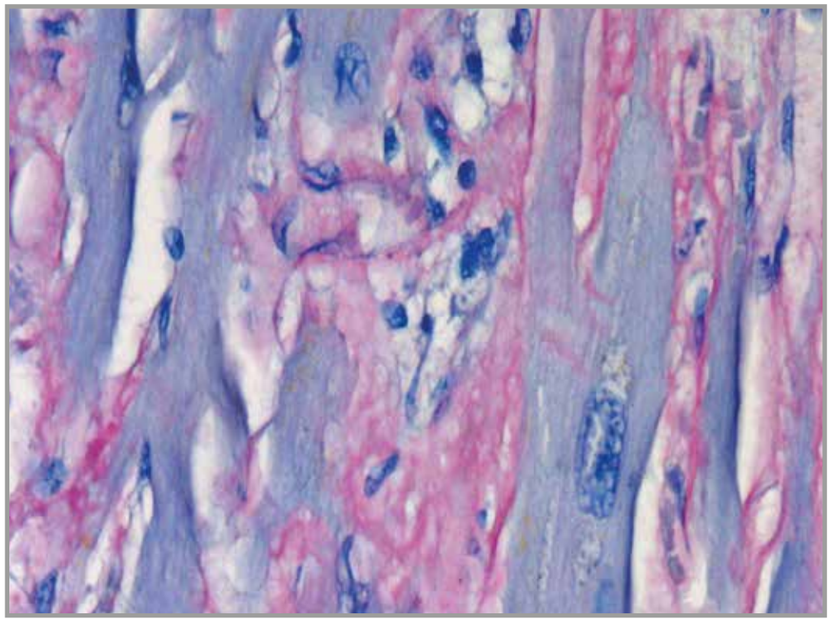
Description
The Kit supplies reagents of Red Sirius stain to highlight amyloid, a colorless, insoluble protein with low molecular weight.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. **Reagent A (Mayer Hematoxylin)** for 3 minutes
3. Wash in running tap water for 5 minutes
4. **Reagent B (Differentiation Alcoholic Buffer)** for 30 seconds
5. Wash in running tap water for 1 minute
6. **Reagent C (Red Sirius)** for 60 minutes
7. Wash in running tap water for 10 minutes
8. 2 quick steps in absolute ethyl alcohol
9. Clear and mount with balsam

Results
Nuclei: Blue-Violet
Amyloid: different shades of Red

Preparation	Paraffin section
Control	Positive case (Amyloidosis)
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	1h 25 min
Suggested fixatives	Formalin
Critical step	None



Connective tissue. Red stain for amyloid.

HISTOLOGICAL STAINING KIT

SIRIUS RED FOR COLLAGEN
code 010254

IVD CE

Description
The Kit supplies reagents of Sirius Red stain to show collagen type I and III. The collagen type I represents the 90% of the total collagen present in connective tissues, bones, skin, tendons. The collagen type III is present in reticular fibers and tissue, skin, smooth muscle.

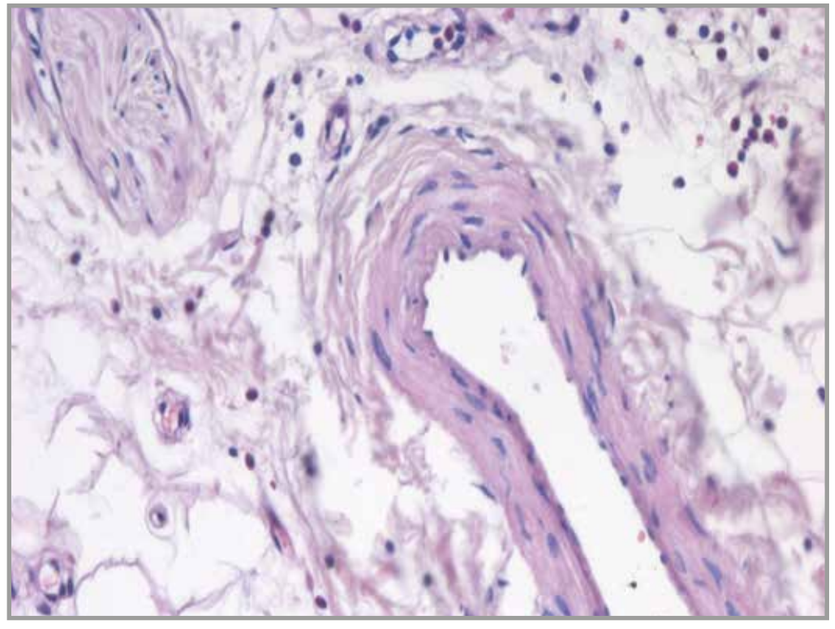
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. **Reagent A (Picric Red Sirius)** for 60 minutes
3. Wash in distilled water
4. **Reagent B (Mayer Hematoxylin)** for 10 minutes
5. Wash in running tap water for 10 minutes
6. 2 quick steps in absolute ethyl alcohol
7. Clear and mount with balsam

Results

Nuclei:	Blue-Violet
Collagen:	different shades of Red
Background, erythrocytes:	Yellow

Preparation	Paraffin section
Control	Tissue with collagen
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	1h 20 min
Suggested fixatives	Formalin
Critical step	None



Skin. Stain of collagen vessels under derma.

HISTOLOGICAL STAINING KIT

TWORT'S STAIN
code 010310

IVD CE

Description
The Kit is intended for use in histological visualization of bacteria. It is a change of Gram stain procedure characterized by a contrast mixture consisting of Neutral red and Fast green to visualize positive (blue-black) and negative (red-pink) Gram bacteria.

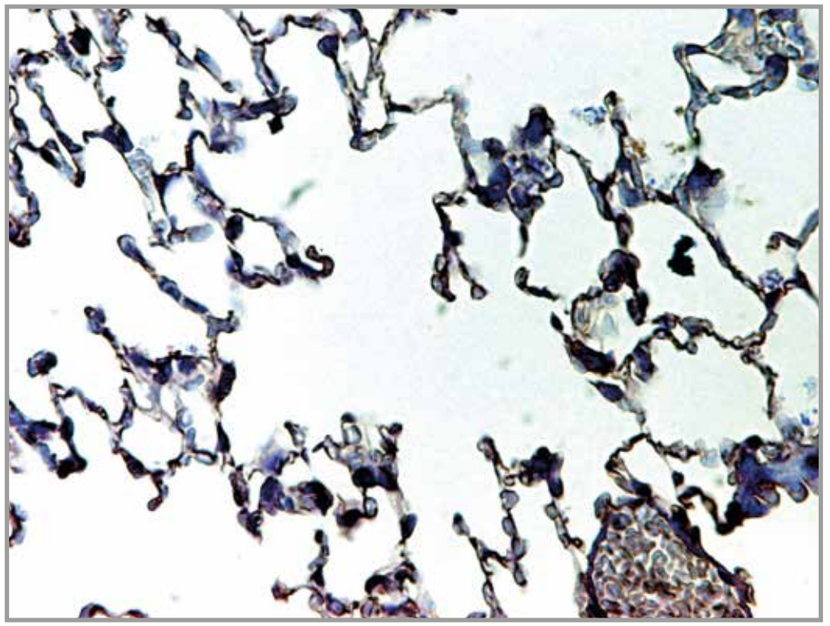
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate section to distilled water
2. **Reagent A (Crystal violet)** for 3 minutes
3. Wash in distilled water
4. **Reagent B (Iodium-Iodide Solution)** for 3 minutes
5. Wash in distilled water
6. Leave it dry in the open air
7. Wash quickly in distilled water
8. Put on the section for 5 minutes Twort solution composed by: **reagent C (Twort A solution)** + 1 ml **reagent D (Twort B solution)** + 30 ml of distilled water
9. Wash quickly in distilled water
10. Dehydrate quickly in absolute ethylic alcohol, clear and mount with balsam

Results

Gram+ bacteria:	Blue-Dark
Gram– bacteria:	Red-Pink
Nuclei:	Red-Violet
Erythrocytes and cytoplasm:	Green
Elastic fibers:	Black

Preparation	Paraffin section
Control	Cecal appendix
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	15 min
Suggested fixatives	Formalin
Critical step	Use Twort solution soon after preparation



Mouse lung. Blue/black stain for bacteria.

HISTOLOGICAL STAINING KIT

VAN GIESON TRICHROME acc. WEIGERT
code 010240

IVD CE

Description
The Kit supplies reagents for Van Gieson Trichrome stain to highlight connective tissue. The connective tissue is highlighted with Picrofuchsin, a solution obtained by mixing two stains: acid fuchsin and picric acid. This solution selectively stains connective tissue, collagen, muscle tissue and thickened epithelium in addition to glial fibrils and cytoplasm.

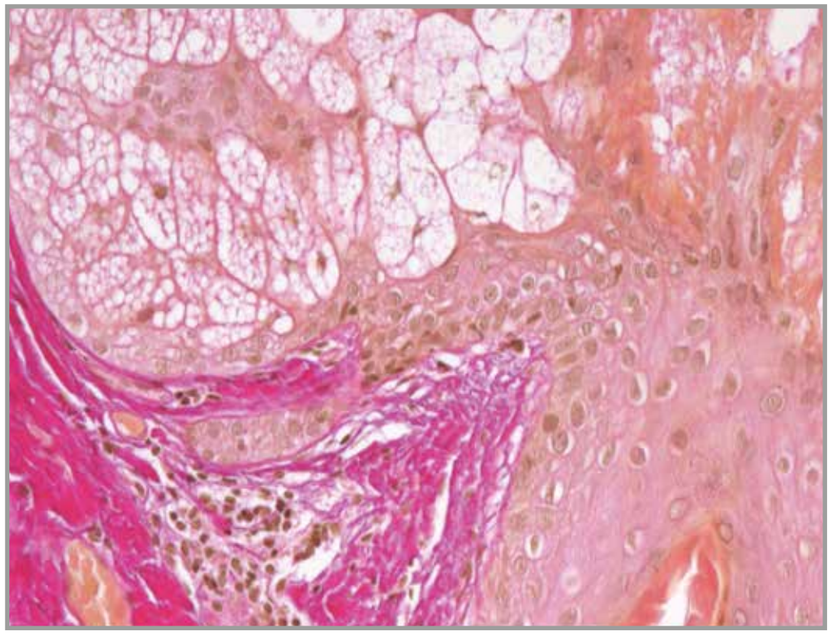
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. Cover the section with 5 drops of **reagent A (Weigert hematoxylin sol. A)** and 5 drops of **reagent B (Weigert hematoxylin sol. B)** for 10 minutes
3. Wash in running tap water for 10 minutes
4. **Reagent C (Picrofuchsin)** for 10 minutes
5. Dehydrate quickly, clear and mount with balsam

Results

Nuclei:	Brown-Black
Collagen:	Red
Cytoplasm, muscle, corneum stratum of the epithelium, erythrocytes:	Yellow

Preparation	Paraffin section
Control	Intestine, liver
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	35 min
Suggested fixatives	Formalin
Critical step	Do not wash in water after reagent C



Skin. Trichrome stain of connective tissue.

HISTOLOGICAL STAINING KIT

VERHOEFF'S STAIN
code 010308

IVD CE

Description
The Kit is intended for use in histological visualization of elastic fibers. It can be used instead to Wiegert's stain. The elastic fibers are visualized very intensely but not selectively by different special stains. The Verhoeff's stain is characterized by a treatment based on iodine and Weigert hematoxylin.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

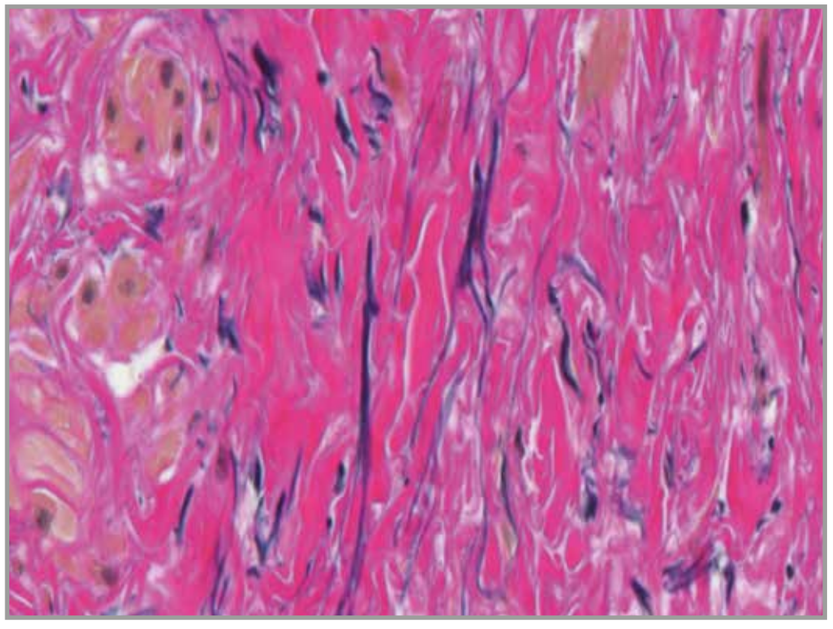
1. Deparaffinize and hydrate section to distilled water
2. Verhoeff solution: 10 drops of **reagent A (Weigert hematoxylin sol. A)** + 10 drops of **reagent B (Weigert hematoxylin sol. B)** + 5 drops of **reagent C (Iodium- Iodide Solution)**
3. Put solution on the section for 30 minutes
4. Wash in distilled water
5. **Reagent D (Differentiation Solution)** for 2 minutes
6. Wash in distilled water
7. **Reagent E (Alcoholic Solution)** for 2 minutes
8. Wash in distilled water
9. **Reagent F (Van Gieson contrast solution*)** for 2 minutes
10. Dehydrate quickly, clear and mount with balsam

* The counterstaining is optional.

Results

Elastic fibers:	Black
Nuclei:	Brown-Black
Collagen*:	Red
Connective tissue*:	Yellow

Preparation	Paraffin section
Control	Aorta, tissue with elastic fibers
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	40 min
Suggested fixatives	Formalin
Critical step	Differentiation step: if too much, do not perform all the staining protocol



Bladder. Black stain of elastic fibers. Counterstaining with Van Gieson.

HISTOLOGICAL STAINING KIT

VON KOSSA acc. McGEE-RUSSEL
code 010241

IVD CE

Description
The Kit is intended for use in histological visualization of calcium. The reaction occurs by replacing calcium ions with silver nitrate and consequent formation of silver phosphate visualized as metallic silver through reducing solution action. The reaction is not specific for calcium but it visualizes anions (phosphate, carbonate, sulfate, oxalate).
Treatment with lithium carbonate prevents staining of uric acid and its salts (false positives). The counterstaining is obtained with Kernechtrot.

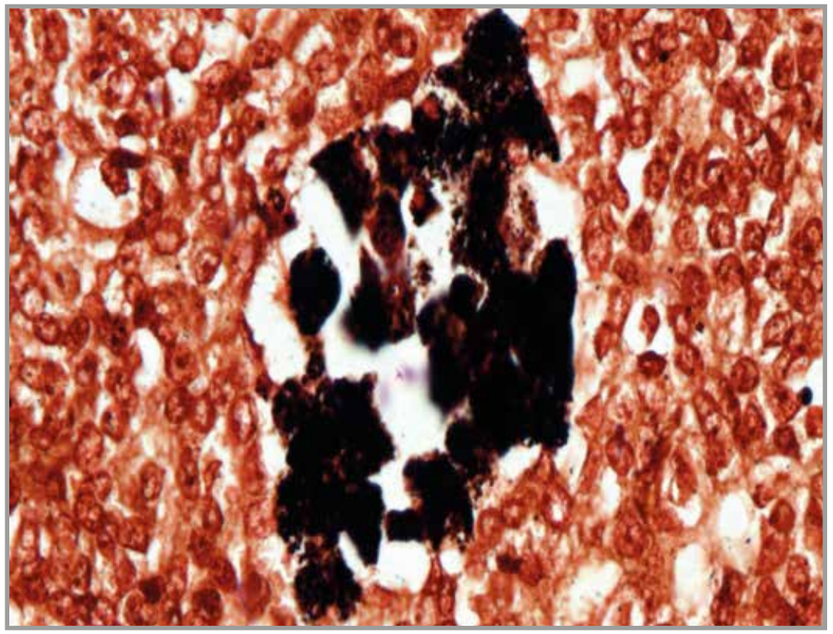
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. **Reagent A (Lithium Carbonate)** for 10 minutes
3. Wash in distilled water
4. **Reagent B (Ammonia solution)** for 60 minutes in the darkness
5. Wash in distilled water 4 times
6. **Reagent C (Reducing Solution)** for 5 minutes (continue if necessary until silver salts become black)
7. Wash in distilled water
8. **Reagent D (Thiosulfate Sodium)** for 5 minutes
9. Wash in distilled water
10. **Reagent E (Kernechtrot)** for 5 minutes
11. Wash in running water for 2 minutes
12. Dehydrate quickly, clear and mount with balsam

Results
Bone and calcium salts: Black
Nuclei and cytoplasm: Pink-Red

WARNING: reagents are stored at +4°C/+8°C, we recommend to keep them at room temperature for at least 10 minutes before the use. If they are used cold, the reaction speed is significantly reduced.

Preparation	Paraffin section
Control	Tissue with calcium deposits
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	1h 40 min
Suggested fixatives	Formalin
Critical step	The step in reagent C



Breast, tumor. Salt deposits shown in black-brown.

HISTOLOGICAL STAINING KIT

WARTHIN STARRY
code 010270

IVD CE

Description
The Kit is intended for use in histological visualization of *Spirochaete Bacteria*, using silver salts.

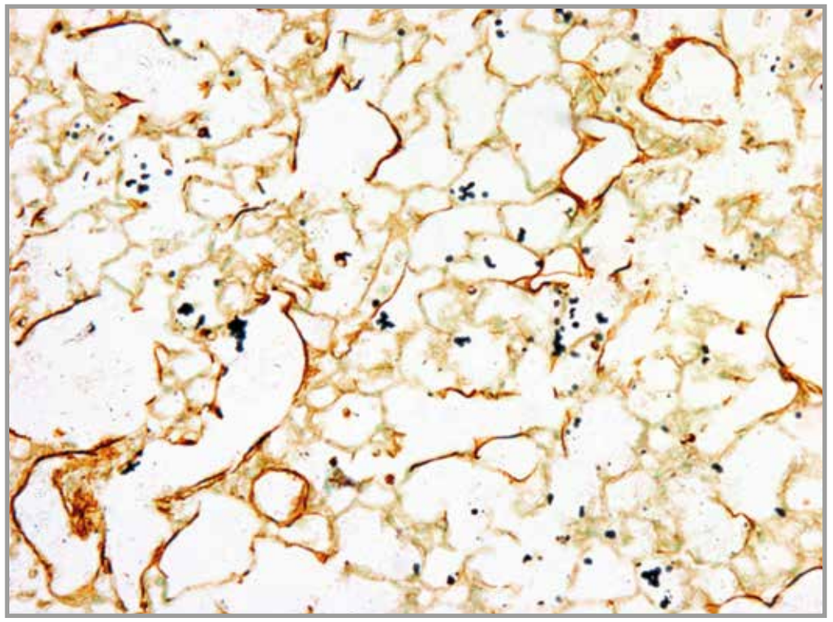
Staining protocol
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water.
2. Melt **reagent A (Citric Acid)** in 1 liter of distilled water. Stir until the complete melting. Verify pH value: between 3.6 and 4.0
3. Melt **reagent B (Silver Nitrate)** in the set solution. Mix with a glass stick (attention DO NOT use metallic objects and use well clean glassware)
4. Pour part of the solution in an histology jar and repair it from the light
5. Immerse slides in the solution (STEP 3) for 40 minutes at +50°C in the darkness
6. Prepare Developing solution:
   - 7 ml of **reagent C (Silver Nitrate)**, heat at +50°C for 10 minutes
   - 18 ml of **reagent D (Gelatine)**, heat at +50°C for 10 minutes
   - 10 ml of **reagent E (Hydroquinone)**, heat at +50°C for 10 minutes
7. Mix the hot reagents in sequence: **C** + **D** + **E** (mix with a glass stick)
8. Pour solution in an histology jar
9. Immerse slides for 3-4 minutes (until the solution turns brown)
10. Wash in hot running tap water
11. Dehydrate quickly, clear and mount with balsam

Results
Spirochetes: Black
Background: Brown-Gold

WARNING: reagents are stored at +4°C/+8°C, we recommend to keep them at room temperature for about 10 minutes before use. If they are used cold, the reaction speed is significantly reduced. The silver solution in STEP 2 is stable for about 12 months if stored at +4°C/+8°C.

Preparation	Paraffin section
Control	Known positive case (*Spirochaetes*)
Storage temperature	+4°C/+8°C
Number of tests	100
Procedure time	1h 20 min
Suggested fixatives	Formalin
Critical step	Solution pH and temperature. Do not use metallic objects, use well clean glassware. Attention: protect reagents from light



Mouse lung. Bacteria shown in black/brown. Gold background.

HISTOLOGICAL STAINING KIT

WEIGERT-VAN GIESON, LONG METHOD
code010218

IVD CE

Description
The Kit supplies reagents to highlight elastic fibers in paraffin sections according to Weigert's stain using Van Gieson as counterstaining to highlight connective tissue.
Staining with resorcin-fuchsin differentiates elastic fibers. Nuclei are shown with Weigert hematoxylin while the Picrofuchsin highlight the connective tissue.
High selectivity due to long time staining protocol.

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

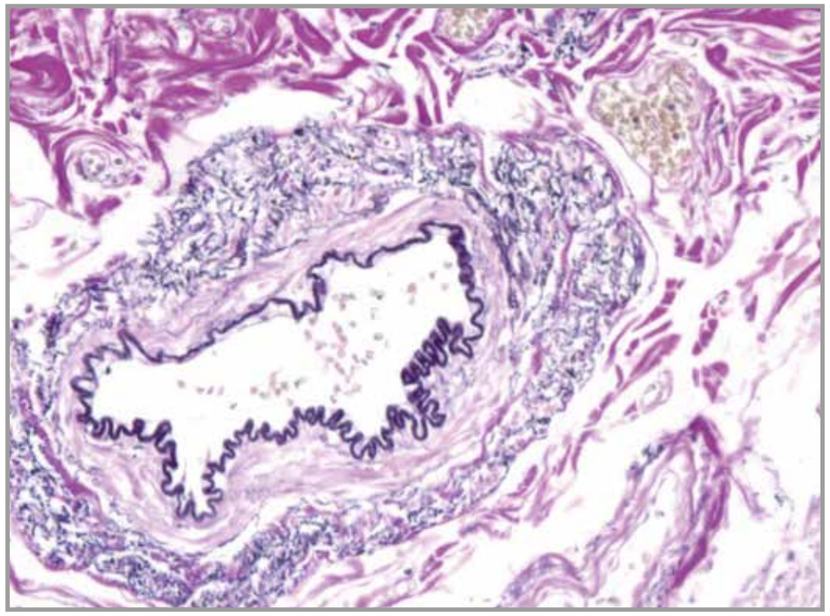
1. Deparaffinize and hydrate to distilled water
2. **Reagent A (Periodic Acid)** for 5 minutes
3. Pour **reagent B (Resorcine Fuchsin)** in a vertical jar for histology and immerse the section (cover the jar or prevent ethyl alcohol evaporation). Incubate overnight in immersion at room temperature (alternatively cover the section and incubate 1 hour at +45°C in incubator box)
4. Wash in distilled water
5. **Reagent C (Jenkins Reagent)** for 10 minutes
6. Wash in distilled water
7. Prepare Weigert hematoxylin: mix 10 drops of **reagent D (Weigert hematoxylin sol. A)** and 10 drops of **reagent E (Weigert hematoxylin sol. B)**
8. Cover the section with Weigert hematoxylin for 8-10 minutes
9. Running tap water for at least 10 minutes
10. **Reagent F (Picrofuchsin)** for 5-7 minutes
11. Wash quickly in distilled water
12. Dehydrate quickly, clear and mount with balsam

WARNING: **reagent B** can be used again after filtration.

Results

Elastic fibers:	from dark Blue to Black
Nuclei:	Black
Collagen:	different shades of Red
Connective tissue and erythrocytes:	Yellow

Preparation	Paraffin section
Control	Skin
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	24h + 40 min with overnight incubation 1h e 40 min with incubation at +45°C
Suggested fixatives	Formalin
Critical step	Avoid section drying



Stomach. Brown-black elastic fibers in arterial vessel. Counterstaining with Van Gieson.

HISTOLOGICAL STAINING KIT

WEIGERT-VAN GIESON, FAST METHOD
code 010243

IVD CE

Description
The Kit supplies reagents to highlight elastic fibers in paraffin sections according to Weigert staining protocol using Van Gieson as counterstaining to highlight connective tissue.
Staining with resorcin-fuchsin differentiates elastic fibers. Nuclei are shown with Weigert hematoxylin while picrofuchsin highlights the connective tissue.
Short time staining protocol different from Kit 010218 "Weigert – Van Gieson long method".

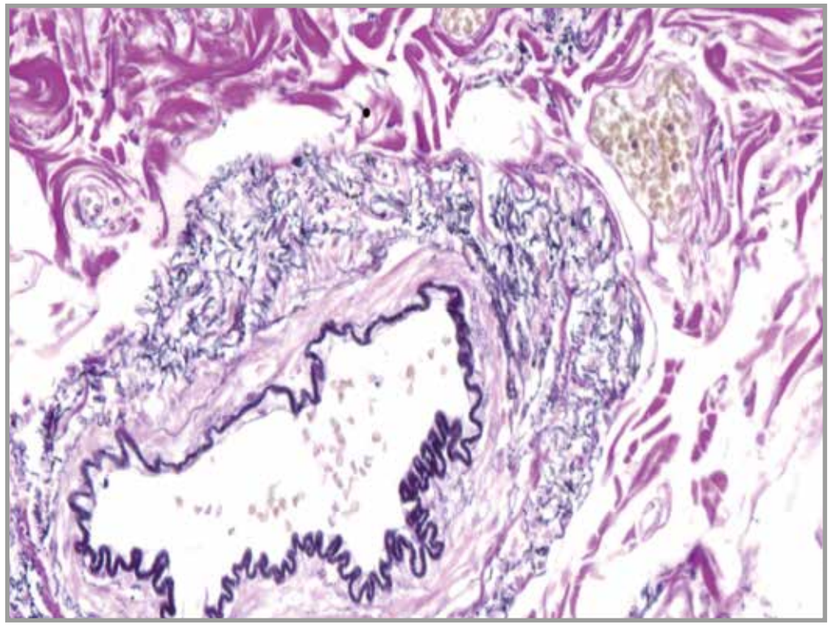
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. Cover the section with 5 drops of **reagent A (Permanganate Potassium)** + 5 drops of **reagent B (Acid Activation Buffer)** for 5 minutes
3. Wash in distilled water
4. **Reagent C (Oxalic Acid)** for 5 minutes
5. Wash in distilled water
6. **Reagent D (Resorcine Fuchsin)** for 30 minutes
7. Wash in distilled water
8. **Reagent E (Jenkins Reagent)** for 2 minutes
9. Wash in distilled water
10. Cover the section with 5 drops of **reagent F (Weigert hematoxylin sol. A)** + 5 drops of **reagent G (Weigert hematoxylin sol. B)** for 10 minutes
11. Wash in running tap water for 10 minutes
12. **Reagent H (Picrofuchsin)** for 10 minutes
13. Wash quickly in distilled water
14. Dehydrate quickly, clear and mount with balsam

Results

Elastic fibers:	From dark Blue to Black
Nuclei:	Black
Collagen:	different shades of Red
Connective tissue, erythrocytes:	Yellow

Preparation	Paraffin section
Control	Skin
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	1h 15 min
Suggested fixatives	Formalin
Critical step	Avoid section drying



Stomach. Brown-black elastic fibers in arterial vessel. Counterstaining with Van Gieson.

HISTOLOGICAL STAINING KIT

WEIGERT FOR ELASTIC FIBERS, LONG METHOD
code 010217

IVD CE

Description
The Kit is designed for demonstrating elastic fibers in paraffin sections.
The step in Permanganate Potassium, followed by Oxalic Acid, increases the staining reaction performances.
The reagent E (Resorcine Fuchsin) allows to differentiate elastic fibers.
High selectivity due to long time staining protocol.

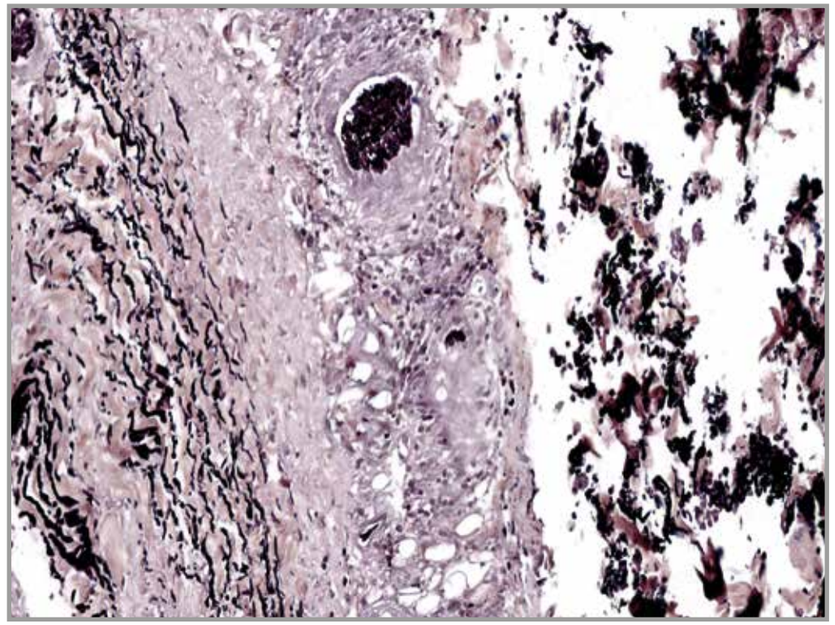
Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. Cover the section with 5 drops of **reagent A (Potassium Permanganate)** + 5 drops of **reagent B (Activation Acid Buffer)**, incubate for 5 minutes
3. Wash in distilled water
4. **Reagent C (Oxalic Acid)** for 5 minutes
5. Wash in distilled water
6. Incubate the slide in overnight immersion with **reagent D (Resorcin Fuchsin)** at room temperature or alternatively cover the section and incubate 1 hour at +45°C in incubator box
7. Wash in distilled water
8. **Reagent E (Jenkins Reagent)** for 10 minutes
9. Wash in distilled water
10. Dehydrate, clear and mount with balsam

WARNING: if used in immersion, **reagent D** can be used again after filtration.

Results
Elastic fibers: from dark Grey to Black

Preparation	Paraffin section
Control	Skin
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	24h + 20 min with overnight incubation 1h 20 min with incubation at +45°C
Suggested fixatives	Formalin
Critical step	Avoid section drying



Connective tissue. Brown-dark stain of elastic fibers.

HISTOLOGICAL STAINING KIT

WEIGERT FOR ELASTIC FIBERS, FAST METHOD
code 010242

IVD CE

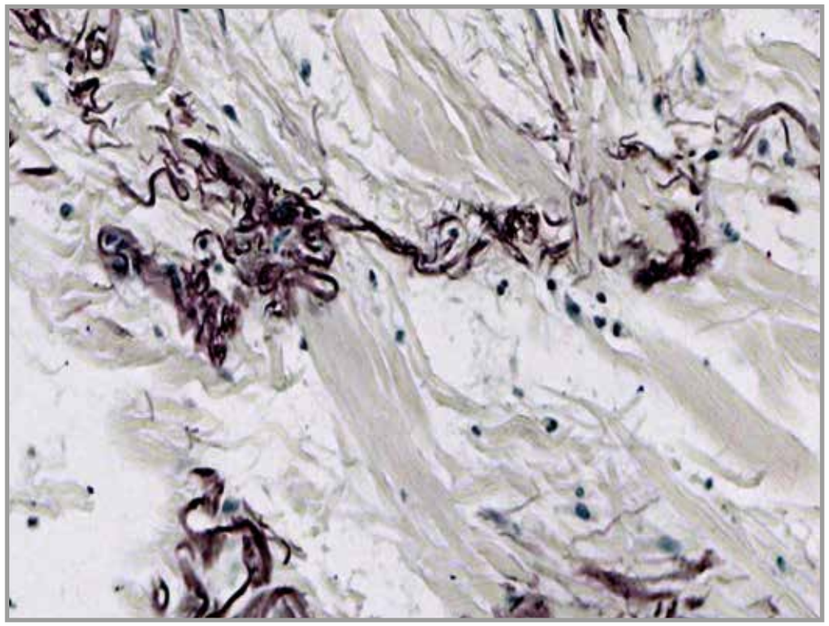
Description
The Kit supplies reagents for Weigert staining protocol with resorcin-fuchsin solution to highlight elastic fibers in tissue section and nuclei counterstaining with Mayer hematoxylin. The step in Potassium Permanganate, followed by Oxalic Acid, increases reaction performances.
Short time staining protocol different from Kit 010217 "Weigert for elastic fibers, long method".

Staining protocol
Drain reagents directly on section in a way to cover it completely.
To avoid section excessive drying, use an incubator box.

1. Deparaffinize and hydrate to distilled water
2. Cover the section with 5 drops of **reagent A (Potassium Permangate)** + 5 drops of **reagent B (Activation Acid Buffer)** for 5 minutes
3. Wash in distilled water
4. **Reagent C (Oxalic Acid)** for 5 minutes
5. Wash in distilled water
6. **Reagent D (Resorcin Fuchsin)** for 30 minutes
7. Wash in distilled water
8. **Reagent E (Jenkins Reagent)** for 2 minutes
9. Wash in distilled water
10. **Reagent F (Mayer Hematoxylin)** for 5 minutes
11. Wash in running tap water for 5 minutes
12. Dehydrate quickly, clear and mount with balsam

Results
Elastic fibers: Purple-Brown
Cell nuclei: Blue-Violet

Preparation	Paraffin section
Control	Skin
Storage temperature	+15°C/+25°C
Number of tests	100
Procedure time	55 min
Suggested fixatives	Formalin
Critical step	Avoid section drying



Connective tissue. Brown-black stain of elastic fibers. No counterstaining.

HEMATOXYLIN

Hematoxylin-eosin staining is the most widely used staining protocol for the study of tissue morphology.

Hematoxylin is a vegetable origin molecule and represents the basis of stains used to show cellular nuclei in histology and cytology. The staining action actually is not due to hematoxylin but to its oxidation product, the hematein.
The oxidation process occurs naturally with exposure to light and air. Since it takes a long time, this process is accelerated by adding chemical oxidant agents.

The hematein has a poor affinity with tissues. For this reason, to the solution is added a chemical mordant able to increase the selectivity of the nuclear staining. Depending on the kind of mordant, there are different kind of hematoxylins: aluminum (or hemallum), ferric and phosphotungstic.
These formulations allow to obtain different cellular nucleus staining shades.

The most commonly used formulations, in histology, are aluminum (or hemallume) ones with mordant based on aluminum (aluminum potassium sulfate or aluminum ammonium sulfate). They have a reddish stain that, after toning in the running water, has a typical blue stain. This kind of hematoxylin, often, requires a bath in acid alcohol (99 ml of ethyl alcohol 70° + 1 ml of hydrochloric acid) to remove non-specific cytoplasm stain.
Due they are very sensitive to acid stains, these hematoxylins are not widely used in special stain, but are widely used in routine stain associated to eosin.

The stain with hematoxylin is very sensitive to pH conditions: for the toning is required running water with a neutral pH because excessively alkaline or chlorinated water may interfere with the staining results.

Hematoxylin/Mayer Hematoxylin – HISTOLOGY

IVD CE

Reagent features: It is a long lasting hematoxylin, red/violet colour, to be used in progressive way.
Oxidizing agent: Sodium Iodate

Code	Packaging
C0302	500 ml
C0303	1 lt
C0305	2.5 lt
C0306	5 lt

Harris Hematoxylin – HISTOLOGY

IVD CE

Reagent features: It is the common hematoxylin characterized by a stain between purple and blue.
Oxidizing agent: Sodium Iodate (firstly mercuric oxide was used, then replaced due to its dangerousness).

Results: Nuclei turn blue/light blue and the stain results very clear.

Code	Packaging
C0282	500 ml
C0283	1 lt
C0285	2.5 lt
C0286	5 lt

Carazzi Hematoxylin - HISTOLOGY

IVD CE

Reagent features: The stain is violet/dark blue and is characterized by glycerol presence that makes this hematoxylin long lasting and increases its selective nucleus stain.
Oxidizing agent: Potassium Iodate

Results: nuclei turn Blue/Violet

Code	Packaging
C0202	500 ml
C0203	1 lt
C0205	2.5 lt
C0206	5 lt

HEMATOXYLIN

Gill Hematoxylin

IVD CE

Reagent features: This hematoxylin is characterized by ethylene glycerol presence.
Oxidizing agent: Sodium Iodate
There are 3 formula containing different hematoxylin concentration (normal, double or triple concentration):

- **Gill I** - normal concentration, recommended for paraffin sections
- **Gill II** - double concentration, recommended for paraffin sections and cytology
- **Gill III** - triple concentration, recommended for paraffin and cryostat sections

Results: The nuclei stain changes from light blue to blue/very dark violet.
Gill I hematoxylin is comparable to Mayer hematoxylin.

Code	Packaging
C0252	500 ml
C0253	1 lt
C0255	2.5 lt
C0256	5 lt

Gill II hematoxylin is comparable to Harris hematoxylin for histology and requires differentiation with acid alcohol.

Code	Packaging
C0262	500 ml
C0263	1 lt
C0265	2.5 lt
C0266	5 lt

Gill III is not widely used in histology in paraffin sections as the nuclear staining is very strong. It is recommended for cryostat due to its quick action.

Code	Packaging
C0272	500 ml
C0273	1 lt
C0275	2.5 lt
C0276	5 lt

HHS Hematoxylin – High Specificity Hematoxylin

IVD CE

Reagent feature: This formula has been designed by Diapath. Its slightly acid pH increases nuclear stain selectivity preventing non-specific cytoplasm stain. Recommended for gastric biopsies.
Oxidizing agent: Potassium Iodate

Results: Nuclei turn blue

Code	Packaging
C0292	500 ml
C0293	1 lt
C0295	2.5 lt
C0296	5 lt

Weigert Hematoxylin

IVD CE

Reagent features: Weigert hematoxylin is a ferric hematoxylin composed by two reagents that are mixed before their use with relationship 1:1. The ready-to-use mixed solution is stable for some weeks, while origin solutions are stable longer. It is recommended to mix origin solutions at least 20 minutes before the use. It is mainly used in special stains (especially trichrome ones). Not used in routine.
Mordant and oxidizing agent: Iron Chloride

Results: nuclei turn black

- **Weigert hematoxylin (reagent A)** (CODE C022X)
- **Weigert hematoxylin (reagent B)** (CODE C023X)

Code	Packaging
C0221	125 ml
C0222	500 ml
C0223	1 lt

Code	Packaging
C0231	125 ml
C0232	500 ml
C0233	1 lt

EOSIN

The hematoxylin-eosin stain is the most used protocol to study tissue morphology.
The eosin is an artificial reagent used as cytoplasmatic stain that, according to cell structure, gives a stain between pink and red. The eosin powder can be dissolved both in water and in ethyl alcohol.

Polychromatic Aqueous Eosin 1%

IVD CE

Reagent features: watery stain, dark red. The addition of acetic acid, before the use (0.5 ml of acetic acid in 1 liter of solution) makes the stain more intense and bright. Its polychromatic stain allows to show tissue structures with a good nuclei/cytoplasm differentiation. Differentiation is important for the good stain performance. It occurs both in water step and in ethyl alcohol step. Usable with all hematoxylin.

Results: cytoplasm turns between Pink and Red

Code	Packaging
C0362	500 ml
C0363	1 lt
C0365	2.5 lt
C0366	5 lt

Alcoholic Eosin 0.5%

IVD CE

Reagent features: Alcohol-based stain, red/bright orange. The alcoholic eosin use allows a staining protocol faster and easier than the aqueous eosin. It doesn't need differentiation in running water. Compatible with all hematoxylins.
The staining with alcoholic eosin is more intense and bright than aqueous eosin. The differentiation must be performed quickly. If too long, the eosin is removed by the tissue. Compatible with hematoxylins.

Results: the cytoplasm turns from Pink to very bright Red-Orange

Code	Packaging
C0352	500 ml
C0353	1 lt
C0355	2.5 lt
C0356	5 lt

Phloxin B Aqueous solution 3%

IVD CE

Reagent features: Dark red, watery stain. The stain is more intense and violaceous than using eosin and has a wide chromatic range. It is often associated to Harris or Gill hematoxylin.
Do not use in association with Carazzi hematoxylin because of nuclei turn purplish and they are less visible if associated with Phloxin B.

Results: tissue turns from Pink to dark-Cyclamen Pink

Code	Packaging
C0401	125 ml
C0402	500 ml

Eosin-Phloxin

IVD CE

Reagent features: Watery stain composed by a mix of alcoholic eosin and phloxin. This stain has the features both of eosin and phloxin giving to tissue a more intense stain than eosin but less using only phloxin. It differentiates excellently tissue components. It is recommended the use in association with Harris or Gill II hematoxylin.

Results: from Pink to bright Red. The muscle tissue and collagen are well differentiated. Erythrocytes are stained in bright red

Code	Packaging
C0342	125 ml
C0343	500 ml

Erythrosine

IVD CE

Reagent features: Watery stain composed by erythrosine powder dissolved in water. It is characterized by a particular bright orange erythrocytes stain. The result is similar to the alcoholic eosin with particularly intense and bright stain. Compatible with all hematoxylin.

Results: The tissue turns from Red to bright Pink

Code	Packaging
C0371	125 ml
C0372	500 ml

SPECIAL STAINING INDEX

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