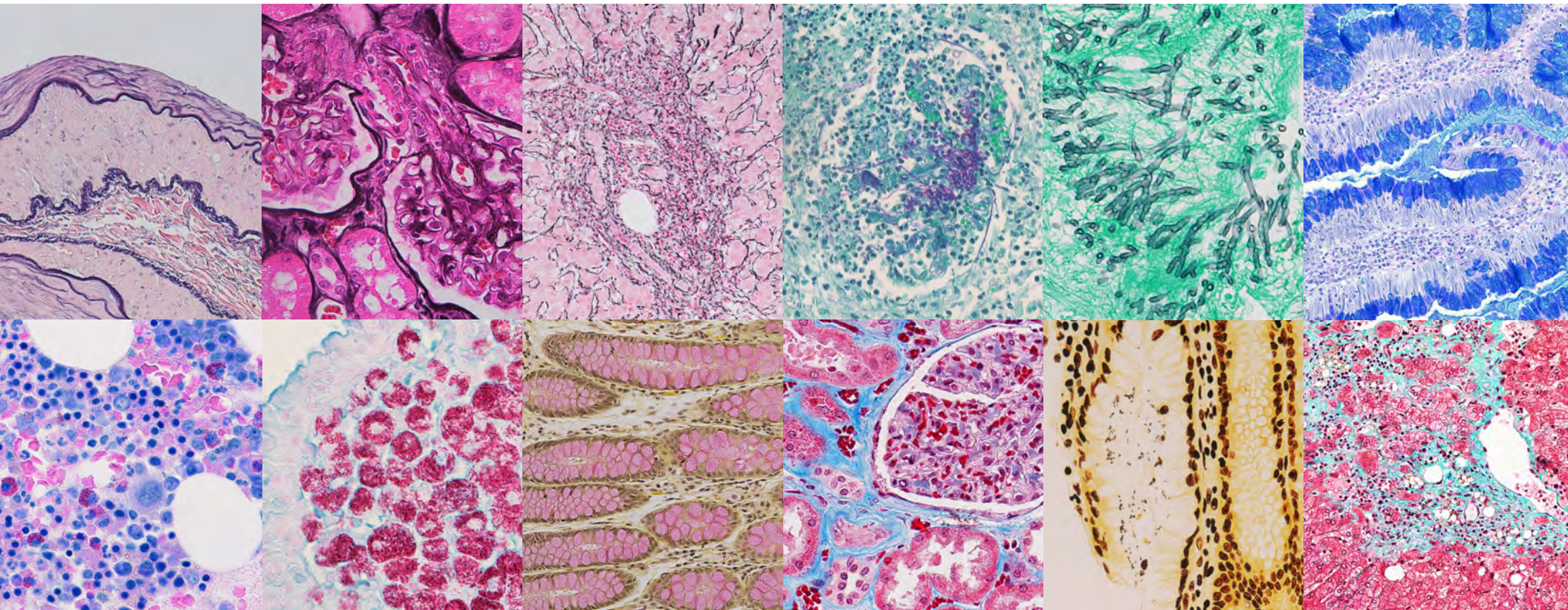


BenchMark Special Stains

Product guide



Quick reference table

Product name	Ordering code	Catalog number	Tissue thickness	Tests per kit	Total vials in kit package	Vials run on instrument	Minimum runtime (minutes) including depar**	Maximum runtime (minutes) including depar**
AFB III Staining Kit	05279437001	860-027	3-5 µm	75 tests	3	3	52	60
Alcian Blue Staining Kit	05279186001	860-002	3-5 µm	75 tests	2	2	44	72
Alcian Yellow Staining Kit	05279321001	860-017	3-5 µm	75 tests	7*	5	68	68
Congo Red Staining Kit	05279429001	860-026	6-10µm	40 tests	3	3	65	85
Diastase Kit	05279208001	860-004	3-5 µm	75 tests	1	1+PAS staining kit	83	110
Elastic Staining Kit	05279216001	860-005	3-5 µm	75 tests	5	5	67	75
Giemsa Staining Kit	05279224001	860-006	3-5 µm	75 tests	1	1	41	41
GMS II Staining Kit	05412749001	860-028	3-5 µm	75 tests	8*	7	124	136
Gram Staining Kit	06890105001	860-039	3-5 µm	75 tests	6	6	80	92
Iron Staining Kit	05279259001	860-009	3-5 µm	75 tests	3	3	48	60
Jones H&E Staining Kit	05279348001	860-019	2-4µm	40 tests	7	7	100	128
Jones Light Green Staining Kit	05279356001	860-020	2-4µm	40 tests	7	7	100	132
Mucicarmine Staining Kit	05279275001	860-011	3-5 µm	75 tests	4	4	56	84
PAS - Alcian Blue	05279194001	860-003	3-5 µm	75 tests	1	1+PAS staining kit	68	84
PAS - Light Green	05279267001	860-010	3-5 µm	75 tests	1	1+PAS staining kit	68	80
PAS Staining Kit	05279291001	860-014	3-5 µm	75 tests	5*	3	69	85
Reticulum II Staining Kit	05279399001	860-024	3-5 µm	75 tests	8	8	112	120
Steiner II Staining Kit	06521894001	860-030	3-5 µm	40 tests	9	8	116	124
Green for Trichrome	06521916001	860-032	3-5 µm	75 tests	1	1+Trichrome staining kit	140	228
Trichrome Staining Kit	06521908001	860-031	3-5 µm	60 tests	7	7	140	228

*Not all vials in the kit are used at one time, e.g., PAS includes three vials of Schiff’s reagent. For product freshness, open one vial at a time.

**All times include 20 minutes for baking (8 minutes as recommended) and depar (12 minutes). If baking offline (i.e. not selected), depar is 12 minutes and not adjustable.

Product name	Ordering code	Catalog number	Format
VENTANA BenchMark Special Stains Deparaffinization Solution (10X)	06523102001	860-036	2 L bottle
BenchMark Special Stains Wash Solution (10X)	06523099001	860-035	2 L bottle
BenchMark Special Stains Liquid Coverslip	06523072001	860-034	2 L bottle
Special Stains Clean Kit	05279313001	860-016	Kit with 50 cleaning cycles
Special Stains Clean Plus	06649327001	860-037	Kit with 50 cleaning cycles
SSR Solution (for use with Gram staining kit)	06890059001	860-038	2 L bottle

General technical notes and post-instrument processing

Post-instrument processing

Standard post processing for most special stains kits

Each lab should validate post-instrument processing steps and timing; however, the following is recommended as a starting point:

1. Rinse in two changes of 95% reagent alcohol (30 seconds each)
2. Dehydrate in three changes absolute reagent alcohol (30 seconds each)
3. Clear in three changes xylene (30 seconds each)
4. Coverslip with a permanent mounting medium

As an alternative to manual post-instrument processing, you may use your VENTANA SYMPHONY automated H&E system by selecting the special stains protocol for fully automated post processing through coverslip.

Giemsa

1. Remove the slides from the instrument and manually differentiate in two changes of 95% alcohol to the desired microscopic preference
2. Dehydrate, clear and coverslip with permanent mounting media

Staining procedure protocol key

Alternative incubation times protocol - An alternative to the Default Protocol, which can offer an appearance which may satisfy users preferences

Default protocol - Staining protocol that is used if the user does not make any changes to the available incubation times or temperatures

Intermediate incubation times protocol - Staining protocol that uses incubation times that fall between the shortest and longest incubation times

Longest incubation times protocol - Staining protocol that uses the longest incubation times available for that assay

Shortest incubation times protocol - Staining protocol that uses the shortest available incubation times for that assay

Alcian Yellow

Product information

BenchMark Special Stains - Alcian Yellow procedure

The Alcian Yellow Staining Kit is used to identify *Helicobacter pylori*.

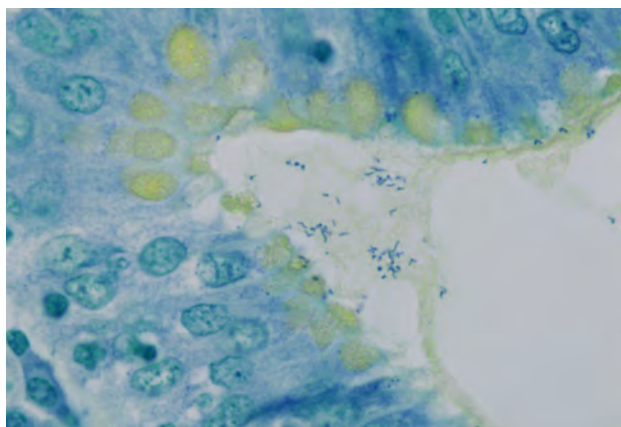


Figure 1. Gastric tissue with *H. Pylori* stained with Alcian Yellow. 1000x.

Alcian Yellow Staining Kit

Catalog number: 860-017

Ordering code: [05279321001](#)

Kit components

1. Alcian Yellow Toluidine Blue contains <1% of the dye
2. Alcian Yellow Stain contains 2.4% alcian yellow, 1.5% acetic acid and 50% ethanol
3. Alcian Yellow Oxidizer contains <1% periodic acid
4. Alcian Yellow Clarifier contains 1.5% sodium metabisulfite and <1% hydrochloric acid
5. Alcian Yellow Sensitizer contains <1% borax and <1% deoxycholic acid

AFB III

Product information

BenchMark Special Stains – AFB III procedure

The AFB III Staining Kit is a modification of the Ziehl-Neelsen stain and Fite stain for acid-fast organisms.¹ A carbol fuchsin solution is used to stain acid-fast organisms and components red. Aniline blue counterstain is applied to provide a contrasting blue background.

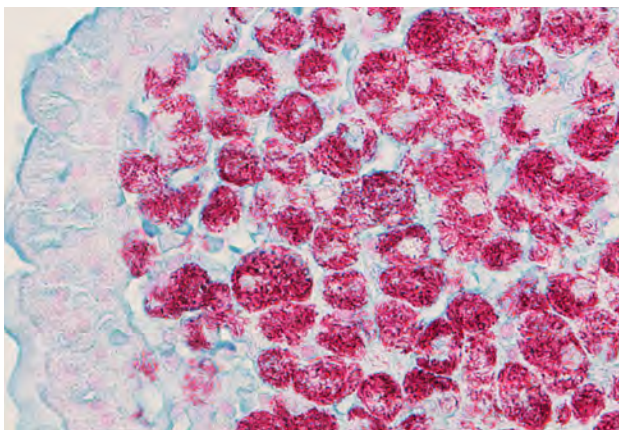


Figure 1. AFB organisms staining on infected colon, 600x.

AFB III Staining Kit

Catalog number: 860-027

Ordering code: [05279437001](#)

Kit components

1. AFB Stain contains proprietary amounts of carbol fuchsin and phenol
2. AFB Decolorizer II contains 18% sulfuric acid and 64% methanol
3. AFB III Blue contains 0.015% aniline blue and 0.25% acetic acid

References

1. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology. 2nd edition. St. Louis, MO: C.V. Mosby Company; 1980:235-237

AFB III

Staining procedures protocol options

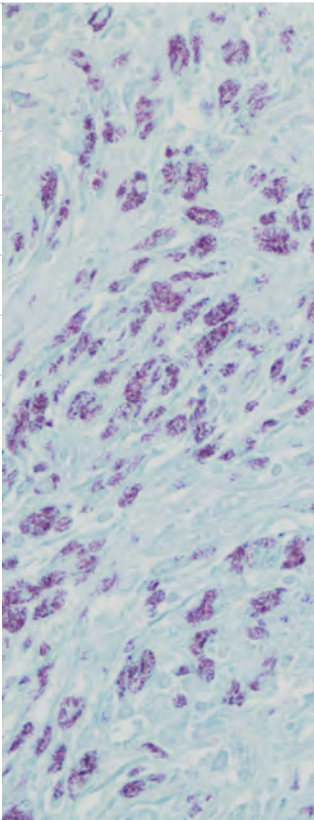
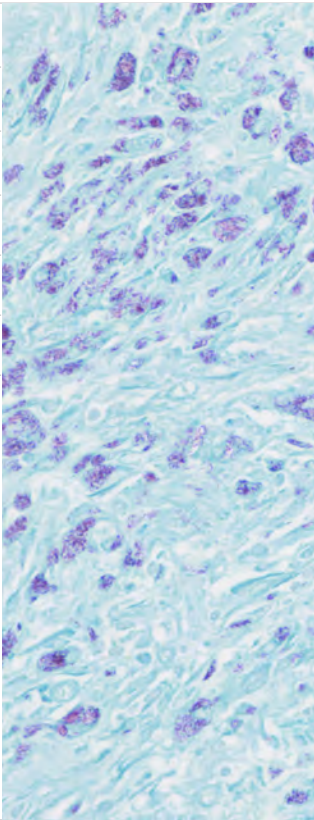
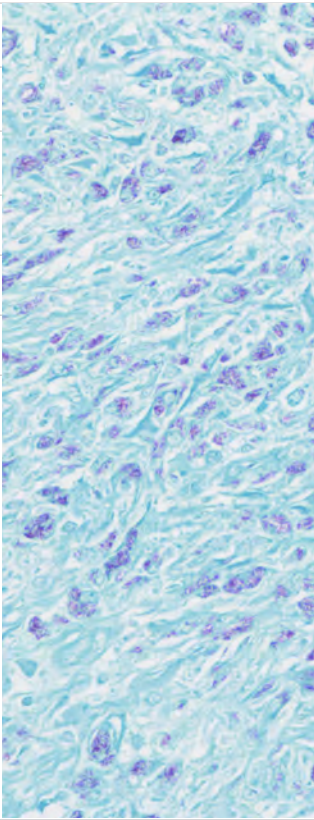
AFB III	Shortest incubation times protocol	Time	Intermediate incubation times protocol	Time	Longest incubation times protocol	Time
Aniline Blue counterstain: 4-12 min		4 min		8 min		12 min

Figure 2 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling. Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

Alcian Blue

Product information

BenchMark Special Stains – Alcian Blue procedure

The Alcian Blue Staining Kit uses the differential pH staining properties of alcian blue to demonstrate acid mucopolysaccharides. At pH 2.5, alcian blue stains sulfated mucins, usually epithelial in origin and usually PAS negative, with some staining in connective tissue (subcutaneous tissue in patients with thyroid deficiency, myxedema and in myxomas). It also stains carboxylated sialomucins, found in the mucins of submaxillary glands, small intestine and upper colon, and sulfated and carboxylated acid mucopolysaccharides.

Alcian Blue Staining Kit

Catalog number: 860-002

Ordering code: [05279186001](#)

Kit components

1. Alcian Blue contains 1.2% alcian blue in a 3% acetic acid solution
2. Nuclear Fast Red Counterstain contains 1% nuclear fast red and 5% aluminum sulfate

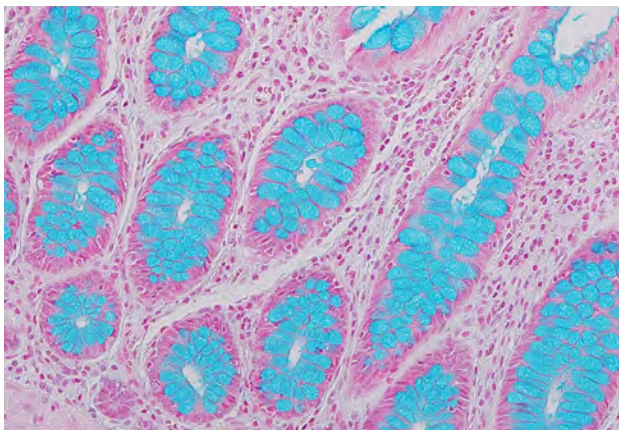


Figure 1. Colon stained with Alcian Blue, 200x.

Alcian Blue

Staining procedures protocol options

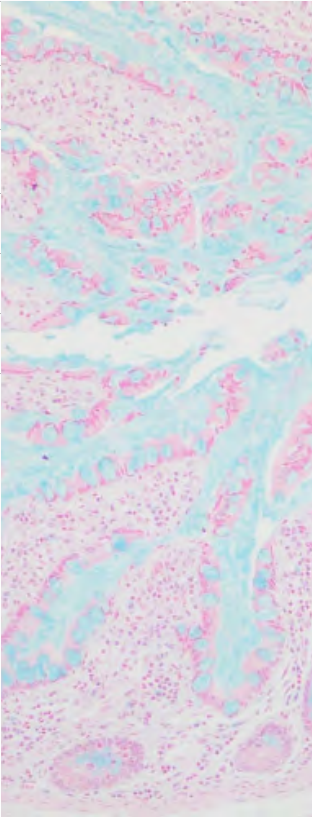
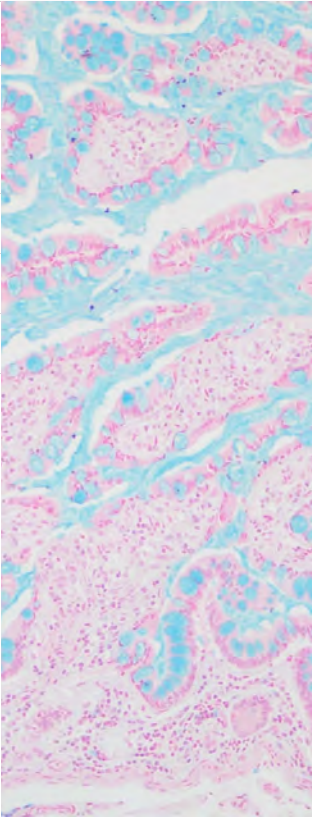
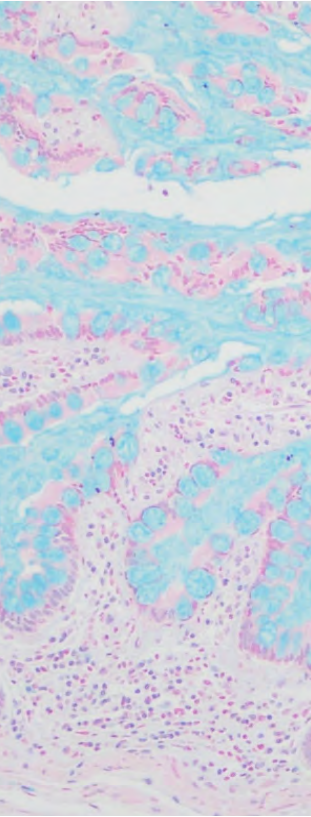
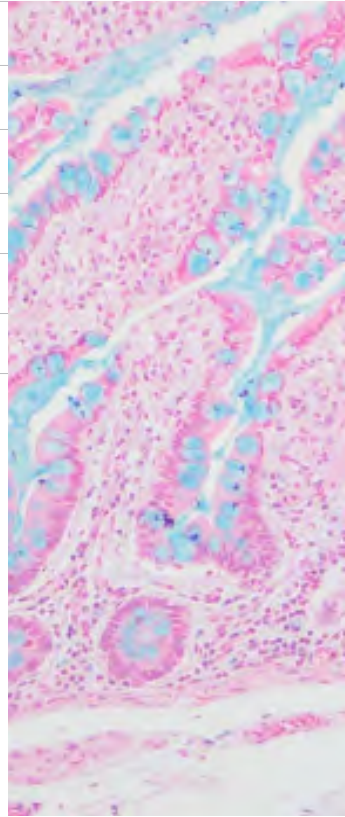
Alcian Blue	Shortest incubation times protocol	Time	Default protocol	Time	Alternative incubation times protocol	Time	Longest incubation times protocol	Time
Alcian Blue: 4-20 min		4 min		8 min		16 min		20 min
NFR counterstain: 4-16 min		4 min		4 min		4 min		16 min

Figure 2 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

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Congo Red

Product information

BenchMark Special Stains - Congo Red procedure

The Congo Red Staining Kit is a modification of Highman's technique.¹ Congo Red Stain is applied to stain amyloid pink to red with an apple green birefringence under polarized light. A Mayer's hematoxylin solution is used to provide contrasting blue nuclear staining.

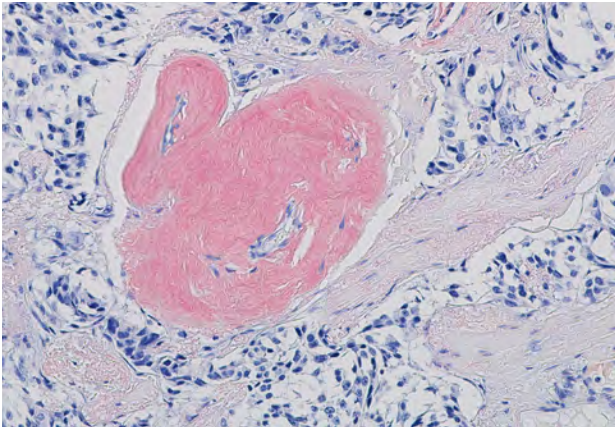


Figure 1. Amyloid in lung stained with Congo Red, regular light microscopy, 200x.

Congo Red Staining Kit

Catalog number: 860-026

Ordering code: [05279429001](#)

Kit components

1. Congo Red Stain contains 1% Congo red and 70% isopropanol
2. Congo Red Buffer contains 0.5% glycine and 2.0% sodium chloride
3. Congo Red Hematoxylin contains modified Mayer's hematoxylin

References

1. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology. 2nd edition. St. Louis, MO: C.V. Mosby Company; 1980:235-237

Congo Red

Staining procedures protocol options

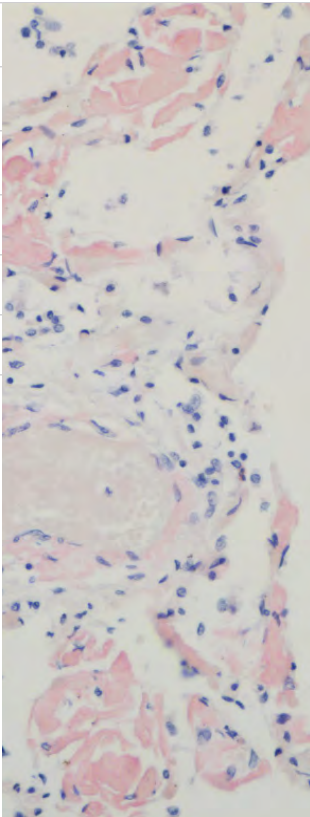
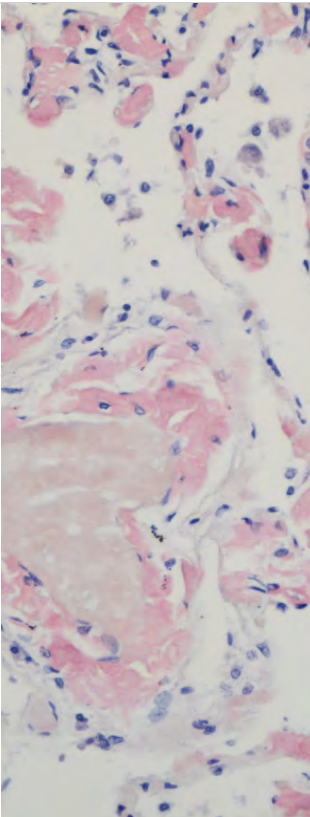
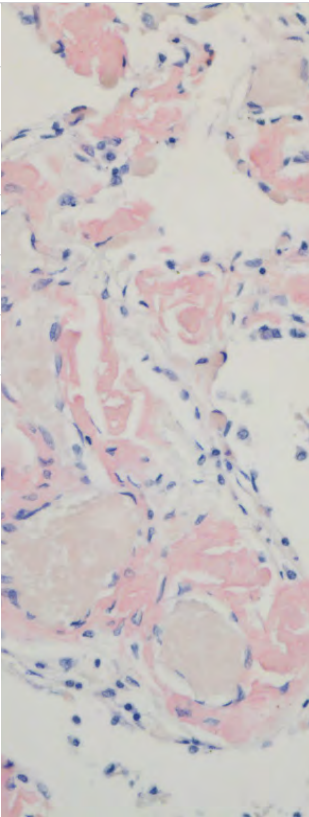
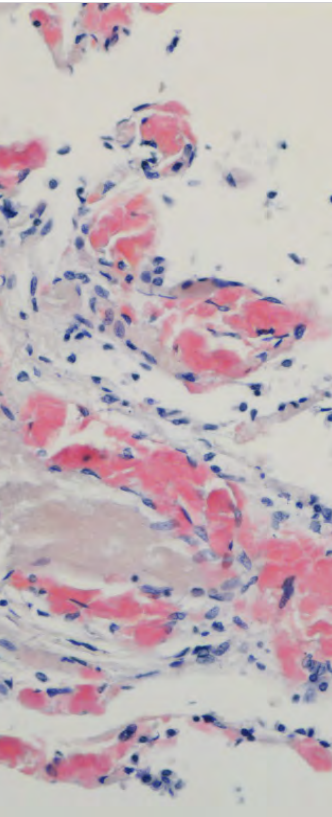
Congo Red Regular light microscopy	Shortest incubation times protocol	Time / temp	Default protocol	Time / temp	Alternative incubation times protocol	Time / temp	Longest incubation times protocol	Time / temp
Reaction temperature: 37-60°C		37°C		37°C		37°C		60°C
Congo Red: 12-20 min		20 min		24 min		32 min		32 min
Hematoxylin: 4-16 min		4 min		12 min		4 min		16 min

Figure 2 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling.
Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

Congo Red

Staining procedures protocol options

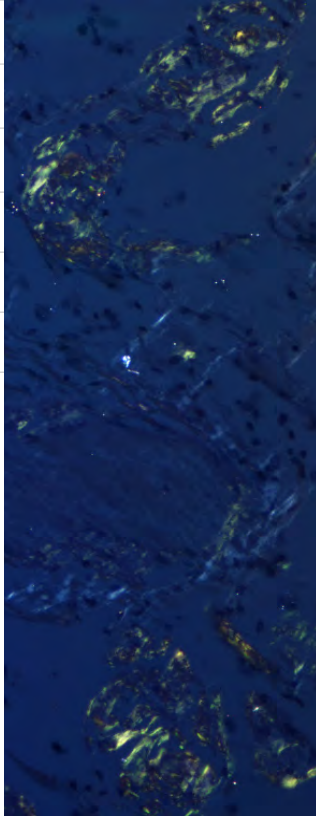
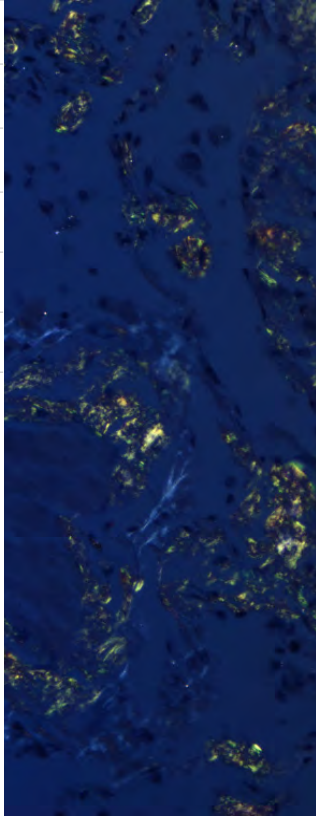
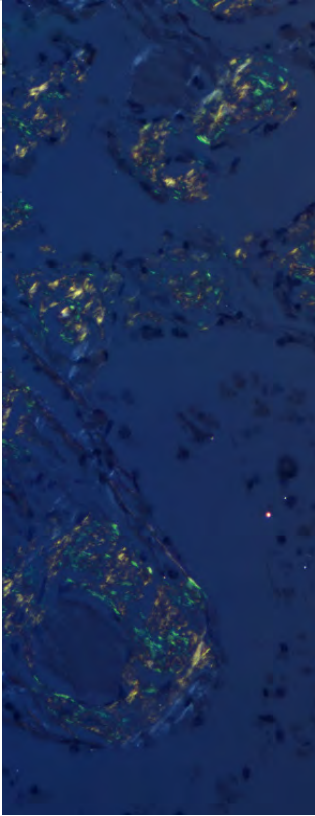
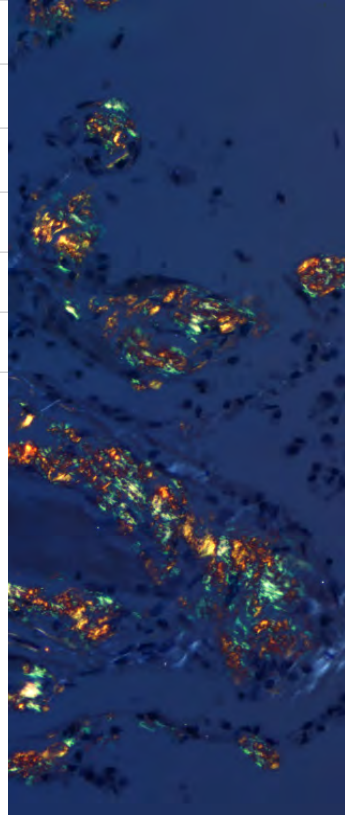
Congo Red Polarized light microscopy	Shortest incubation times protocol	Time / temp	Default protocol	Time / temp	Intermediate incubation times protocol	Time / temp	Longest incubation times protocol	Time / temp
Reaction temperature: 37-60°C		37°C		37°C		37°C		60°C
Congo Red: 12-20 min		20 min		24 min		32 min		32 min
Hematoxylin: 4-16 min		4 min		12 min		4 min		16 min

Figure 3 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

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Elastic

Product information

BenchMark Special Stains - Elastic procedure

The Elastic Staining Kit is a modification of Hart's method for elastic fibers. A resorcin-fuchsin solution is used to stain elastic fibers dark bluish purple to black.¹ Van Gieson's solution is applied to provide contrasting yellow background tissue while staining the collagen pinkish red. This stain is useful in demonstrating atrophy of elastic fibers in cases of emphysema, as well as the thinning and loss of elastic fibers in arteriosclerosis and other vascular diseases.

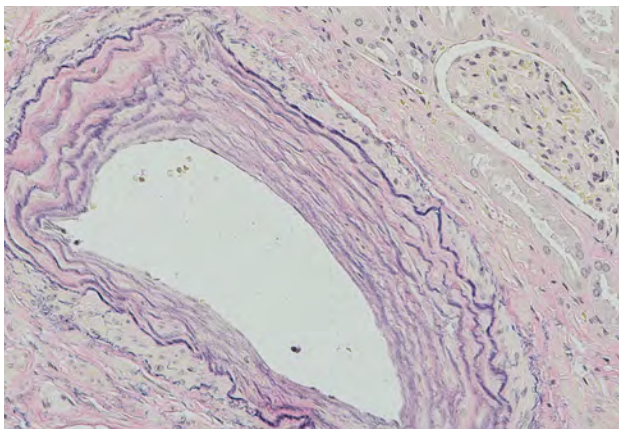


Figure 1. Artery stained with Elastic stain

Elastic Staining Kit

Catalog number: 860-005

Ordering code: [05279216001](#)

Kit components

1. Oxidizer contains less than 1% potassium permanganate
2. Decolorizer contains less than 1% oxalic acid
3. Elastic Tissue Stain contains 66.5% absolute ethanol, 1% hydrochloric acid and 0.8% resorcin fuchsin
4. Elastic Clarifier contains 50% absolute alcohol
5. Van Gieson's solution contains 1.3% picric acid saturated solution

References

1. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology. 2nd edition. St. Louis, MO: C.V. Mosby Company; 1980:235-237

Elastic

Staining procedures protocol options

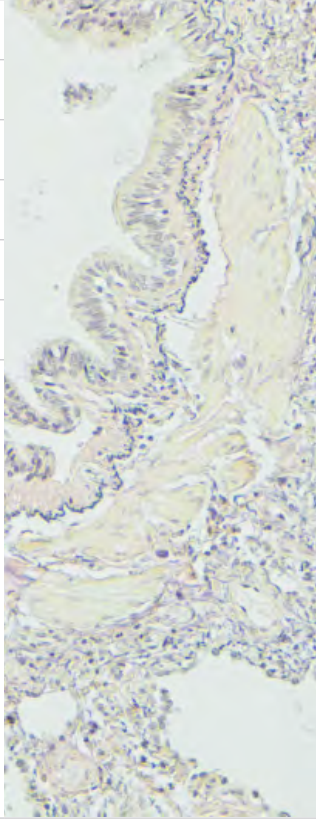
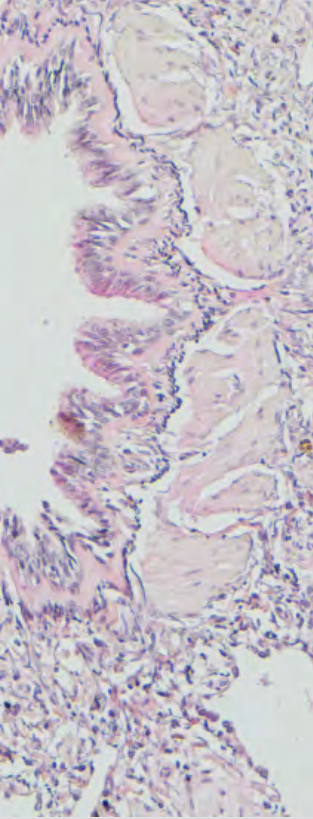
Elastic	Shortest incubation times (and default) protocol	Time	Longest incubation times protocol	Time
Hematoxylin: 8-16 min		8 min		16 min

Figure 2 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling.
Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

Giemsa

Product information

BenchMark Special Stains - Giemsa procedure

The Giemsa Staining Kit is a modification of the original Giemsa stain.¹ Buffered thiazine eosinate solution is used to stain cells differentially with a characteristic blue or pink color.²

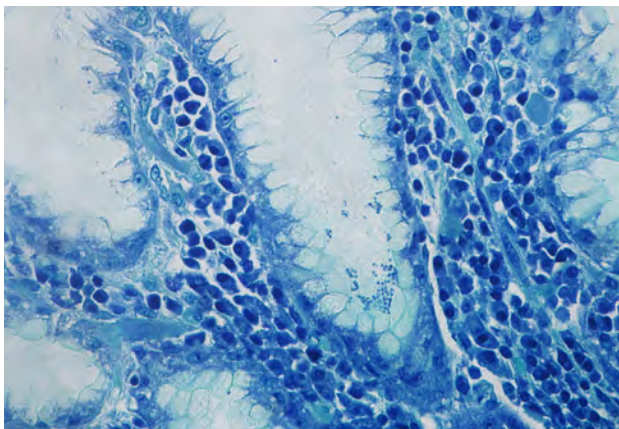


Figure 1. Gastric biopsy stained with Giemsa demonstrating H. Pylori organisms, 600x.

Giemsa Staining Kit

Catalog number: 860-006

Ordering code: [05279224001](#)

Kit components

1. Giemsa Stain contains 0.4% modified Giemsa stain in 70% methanol

References

1. C1. Bancroft and Stevens. Theory and Practice of Histological Techniques, 2nd edition. Edinburgh: Churchill-Livingston, 1982.
2. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
3. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology, 2nd edition. St. Louis, MO: C.V. Mosby Company; 1980:154-156.

Giemsa

Product information

BenchMark Special Stains Giemsa procedure

The Giemsa Staining Kit is a modification of the original Giemsa stain.² Buffered thiazine eosinate solution is used to stain cells differentially with a characteristic blue or pink color.³

Kit components:

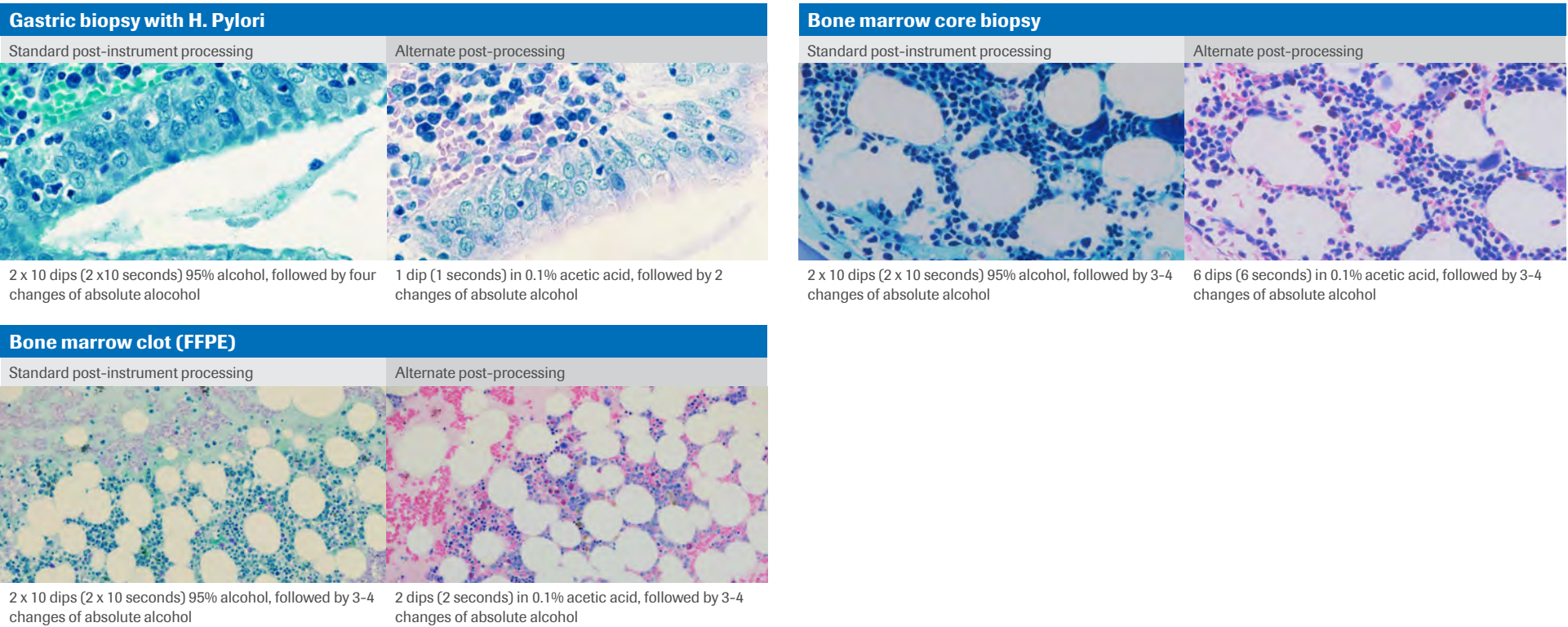
Giemsa stain contains 0.4% modified Giemsa stain in 70% methanol.

Giemsa Staining Kit:

Catalog number 860-006
Ordering code [05279224001](#)

Staining procedures protocol options

Figure 2.



This field guide is intended to be an educational supplement, not a substitute for product labeling.
Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

GMS II

Product information

BenchMark Special Stains - GMS II procedure

The GMS II Staining Kit is a modification of Gomori's Methenamine Silver procedure.¹ This stain has also been modified by Grocott, and is sometimes called Grocott's Methenamine Silver.²

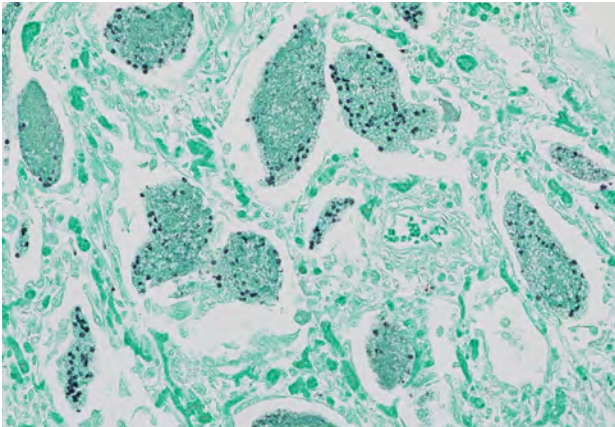


Figure 1. Pneumocystis organisms in lung stained with GMS, 200x.

GMS II Staining Kit

Catalog number: 860-028

Ordering code: [05412749001](#)

Kit components

1. GMS II Oxidizer contains <6% chromium trioxide
2. GMS II Neutralizer contains <1% sodium bisulfite
3. GMS II Silver A contains <1% silver nitrate
4. GMS II Silver B contains <2% sodium borate and <15% Methenamine
5. GMS II Toner contains <1% gold chloride
6. GMS II Fixer contains <3% sodium thiosulfate
7. GMS II Light Green Counterstain contains <1% light green SF yellowish and <1.5% acetic acid

References

1. Bancroft JD, Gamble, M. Theory and Practice of Histological Techniques. 2nd ed. Edinburgh: Churchill-Livingston; 1982
2. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology. 2nd ed. St. Louis, MO: C.V. Mosby Company; 1980

GMS II

Staining procedures protocol options

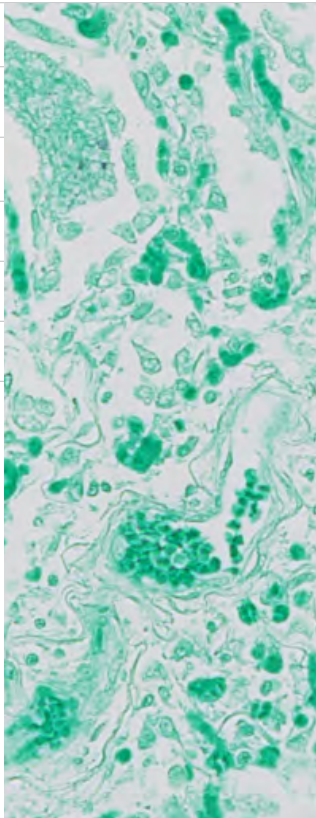
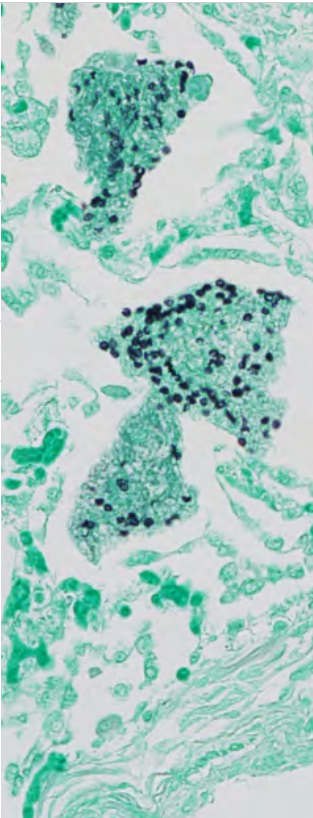
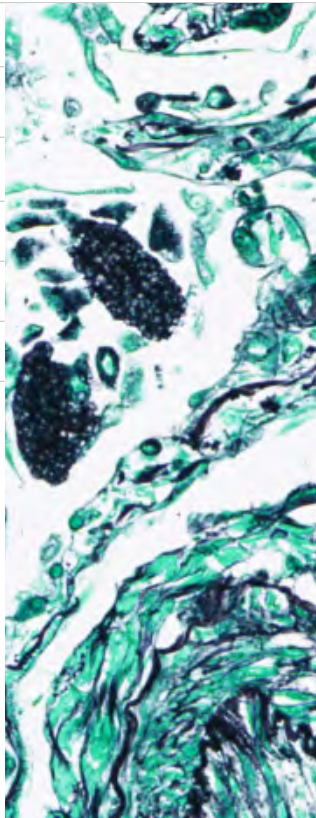
GMS II	Shortest incubation times protocol	Time / temp	Intermediate incubation times protocol	Time / temp	Darkest incubation times protocol	Time / temp
Reaction temperature: 50-60°C		50°C		52°C		60°C
Silver B: 8-16 min		8 min		12 min		16 min
Green counterstain: 4-16 min		4 min		4 min		16 min

Figure 2 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling. Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

Gram

Product information

BenchMark Special Stains - Gram procedure

The Gram Staining Kit is a modification of the original Gram stain.¹⁻³ Gram-negative bacteria stain pink to red and gram-positive bacteria stain blue to dark purple.

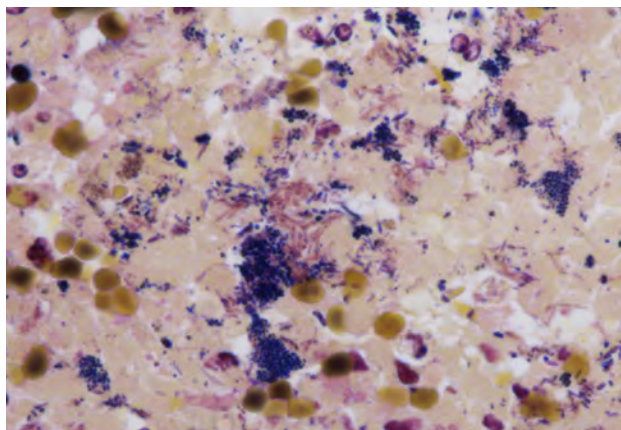


Figure 1. Gram stain with Tartrazine counterstain showing a mixture of Gram-positive and Gram-negative microorganisms, 1000x. (oil)

Gram Staining Kit

Catalog number: 860-039

Ordering code: [06890105001](#)

Kit components

1. Gram Crystal Violet contains 1% crystal violet, 0.7% ammonium oxalate monohydrate, and 17.5% reagent alcohol
2. Gram Iodine contains stabilized gram iodine
3. Gram Basic Fuchsin contains 0.15% basic fuchsin
4. Gram Gallego contains 3% formaldehyde and 1.5% acetic acid
5. Gram Tartrazine contains 0.1% tartrazine and 0.25% acetic acid
6. Gram Fast Green contains 0.002% fast green and 0.025% acetic acid

References

1. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009
2. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology. 2nd ed. St. Louis, MO: C.V. Mosby Company; 1980
3. Bancroft JD, Gamble, M. Theory and Practice of Histological Techniques. 2nd ed. Edinburgh: Churchill-Livingston; 1982

Gram

Staining procedures protocol options

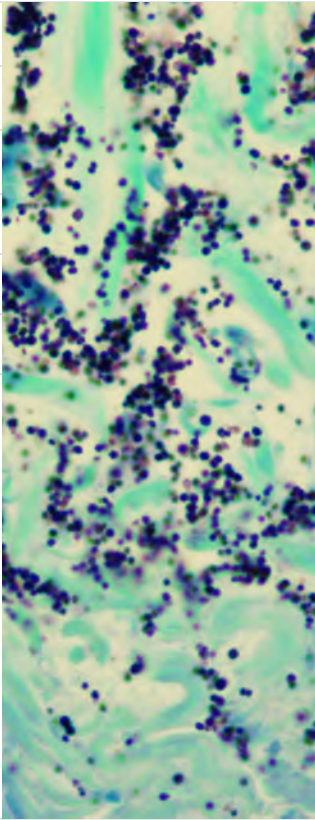
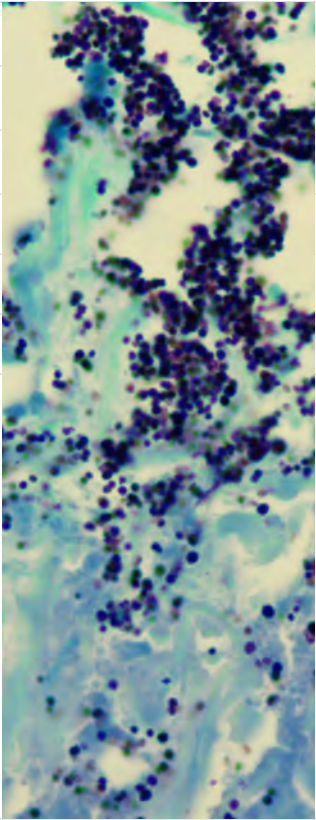
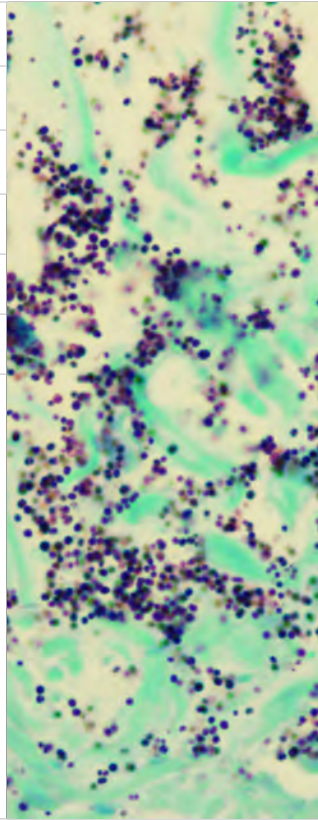
Gram Green Counterstain	Shortest incubation times protocol	Time	Default protocol	Time	Longest incubation times protocol	Time
Optimize Gram-positive: 4–16 min		4 min		8 min		12 min
Optimize Gram-negative: 4–12 min		4 min		8 min		16 min

Figure 2 - Sample images of staining achieved with different protocol time selections. Appendix, showing gram positive bacteria only.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling.
Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

Gram

Staining procedures protocol options

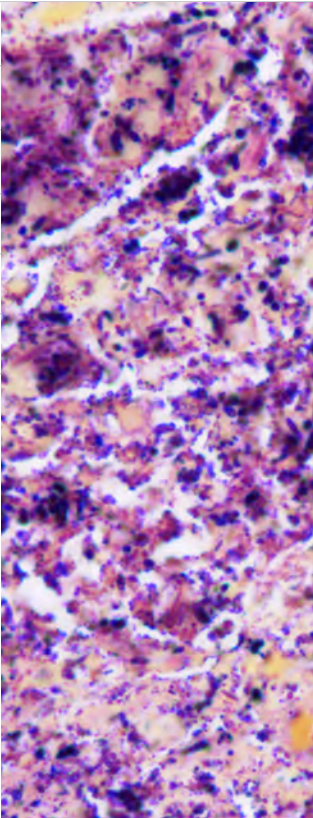
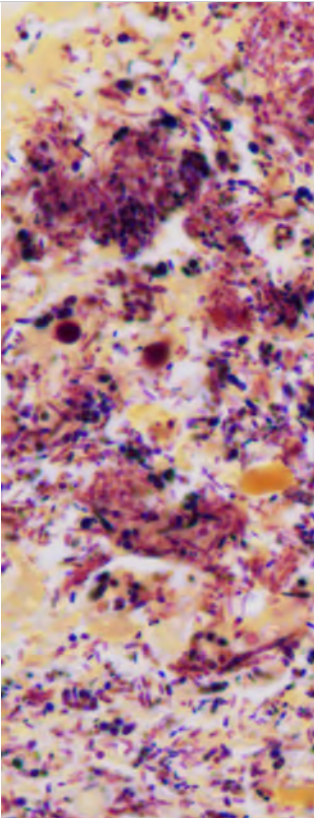
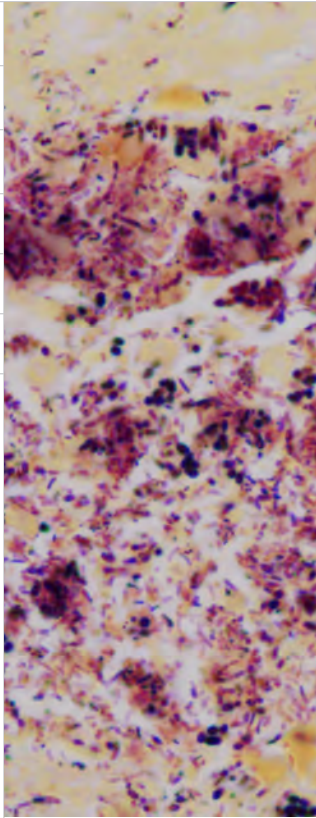
Gram Yellow Counterstain	Shortest incubation times protocol	Time	Default protocol	Time	Longest incubation times protocol	Time
Optimize Gram-positive: 4–16 min		4 min		8 min		16 min
Optimize Gram-negative: 4–12 min		4 min		12 min		12 min

Figure 3 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling.
Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

Iron

Product information

BenchMark Special Stains - Iron Staining procedure

This iron stain is based on the historic Prussian blue reaction, which has previously been modified by Gomori, Perls and Mallory.¹⁻² In the Iron Staining Kit, Iron Reagent A and Iron Reagent B create an acidic ferrocyanide, which reacts with ionic iron in the tissue to produce a bright blue color. Nuclear Fast Red counterstain: is applied to provide a contrasting pink to red background.

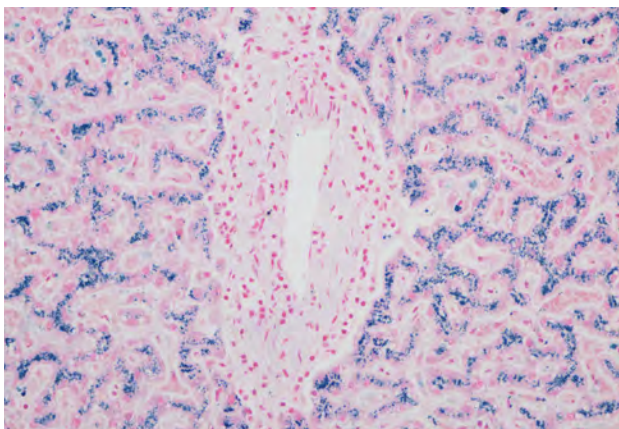


Figure 1. Liver with iron deposits stained with Iron Stain , 200x.

Iron Staining Kit

Catalog number: 860-009

Ordering code: [05279259001](#)

Kit components

1. Iron Reagent A contains 10% potassium ferrocyanide
2. Iron Reagent B contains less than 2% hydrochloric acid
3. Nuclear Fast Red Counterstain contains less than 1% nuclear fast red and 5% aluminum sulfate

References

1. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology. 2nd ed. St. Louis, MO: C.V. Mosby Company; 1980
2. Bancroft and Stevens. Theory and Practice of Histological Techniques, 2nd edition. Edinburgh: Churchill-Livingston; 1982

Iron

Staining procedures protocol options

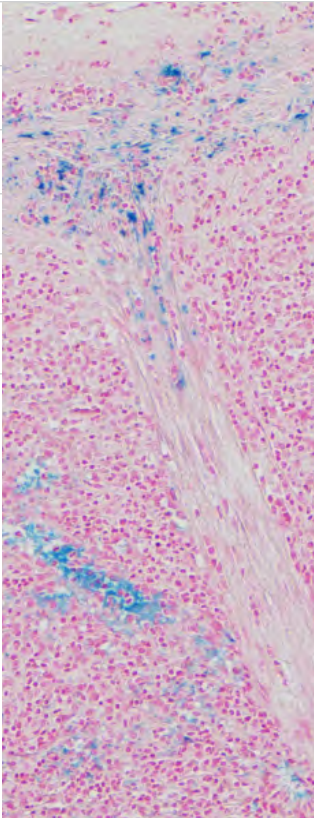
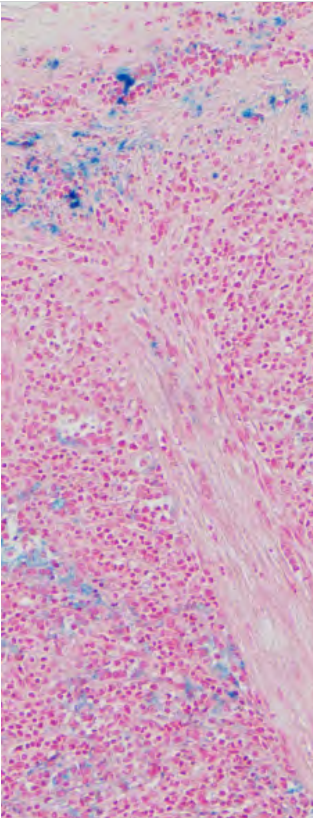
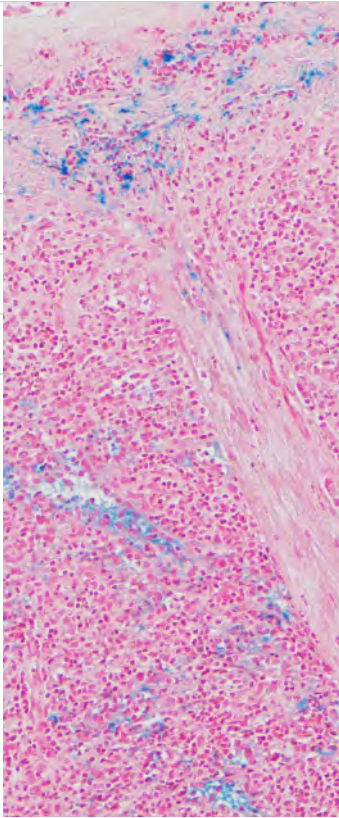
Iron	Shortest incubation times (and optimized) protocol	Time	Intermediate incubation times protocol	Time	Longest incubation times protocol	Time
NFR counterstain: 4-16 min		4 min		12 min		16 min

Figure 2- Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling.
Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

Jones H&E & Jones Light Green

Product information

BenchMark Special Stains - Jones Staining procedure

The Jones Staining Kit is a modification of Jones Methenamine Silver procedure.¹ Periodic acid is used to oxidize carbohydrates to aldehyde groups. The combined Jones Silver A and Jones Silver B solutions form a methenamine-silver complex that is easily reduced to metallic silver by the aldehyde groups. Toner reagent contains gold chloride to form a more stable gold complex and remove the yellow tones from the tissue. Fixer, with thiosulfate, stops the reaction and removes any unreduced silver from the section. Two different types of counterstain: (Hematoxylin & Eosin, and Light Green) are available to provide contrasting background to the silver stain.

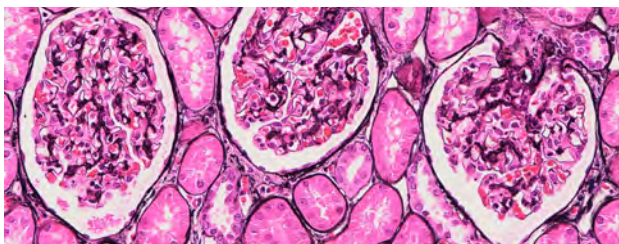


Figure 1. Kidney stained with Jones using Hematoxylin and Eosin counterstain, 200x. H&E

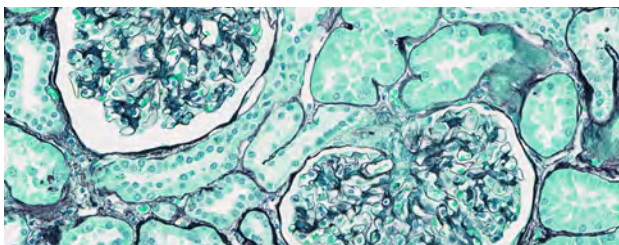


Figure 2. Kidney stained with Jones using Light Green counterstain, 200x.

Jones Staining Kit

Catalog number: 860-019

Ordering code: 05279348001

Kit components

1. Jones Periodic Acid contains 1% periodic acid
2. Jones Silver A contains 1% silver nitrate
3. Jones Silver B contains <2% sodium borate and less than 14% methenamine
4. Toner contains <1% gold chloride
5. Fixer contains 2% sodium thiosulfate
6. Jones Hematoxylin contains modified Mayer's Hematoxylin (contains sodium iodate and ethylene glycol)
7. Jones Eosin contains <1% Eosin Y in an alcohol solution
8. Jones Periodic Acid contains 1% periodic acid

Jones Light Green Staining Kit

Catalog number: 860-020

Ordering code: 05279356001

Kit components

1. Jones Periodic Acid contains 1% periodic acid
2. Jones Silver A contains 1% silver nitrate
3. Jones Silver B contains <2% sodium borate and less than 14% methenamine
4. Toner contains <1% gold chloride
5. Fixer contains 2% sodium thiosulfate
6. Jones Hematoxylin contains modified Mayer's Hematoxylin (contains sodium iodate and ethylene glycol)
7. Jones Light Green contains <1.09% Light Green Y in acetic acid
8. Jones Periodic Acid contains 1% periodic acid

References

1. Koski JP. Silver methenamine-borate (SMB): Cost reduction with technical improvement in silver nitrate-gold chloride impregnations. J Histotechnol. 1981;3:115

Jones H&E

Staining procedures protocol options

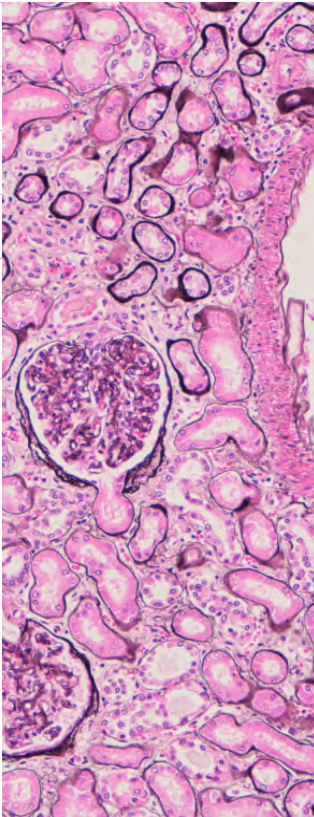
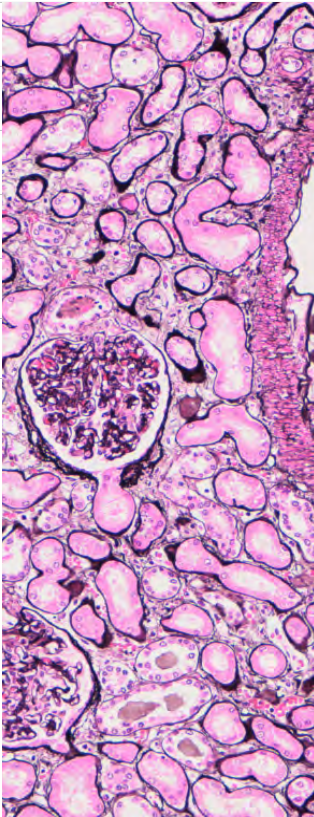
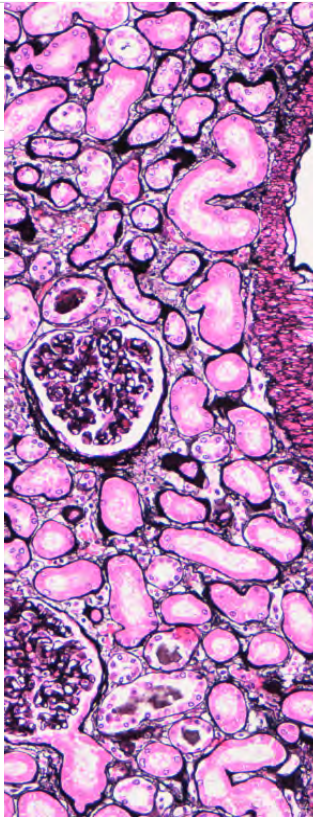
Jones H&E	Shortest incubation times protocol	Time / temp	Default protocol	Time / temp	Longest incubation times protocol	Time / temp
Reaction temperature: 55-60°C		55°C		60°C		60°C
Silver: 8-20 min		8 min		12 min		20 min
Hematoxylin: 8-16 min		8 min		8 min		16 min
Eosin: 4-12 min		4 min		8 min		12 min

Figure 3 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling. Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

Jones Light Green

Staining procedures protocol options

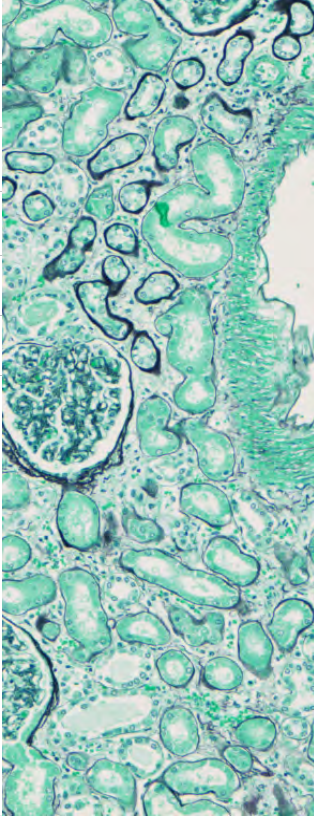
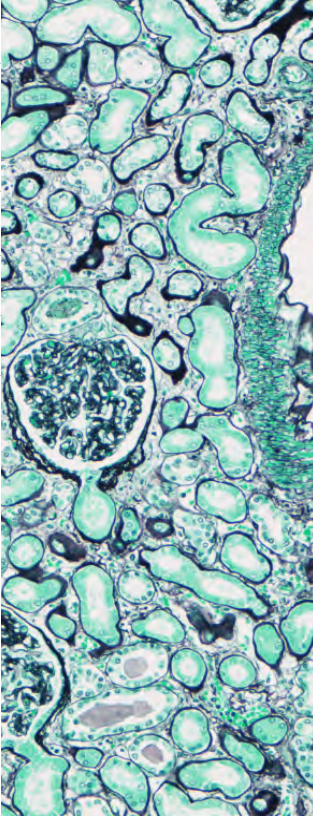
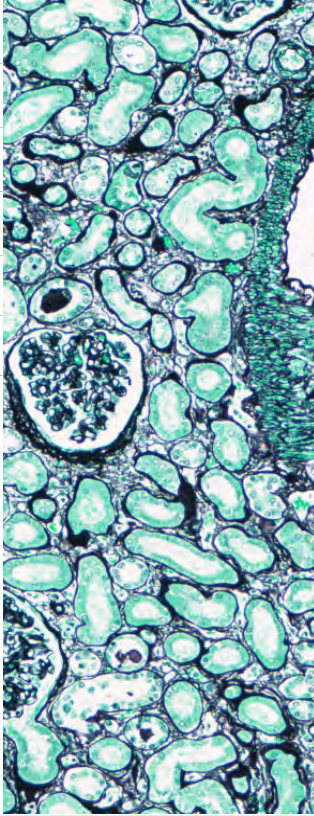
Jones Light Green	Shortest incubation times protocol	Time / temp	Default protocol	Time / temp	Longest incubation times protocol	Time / temp
Reaction temperature: 55-60°C		55°C		60°C		60°C
Silver: 8-20 min		8 min		12 min		20 min
Hematoxylin: 8-16 min		8 min		12 min		16 min
Light Green: 4-16 min		4 min		8 min		16 m

Figure 4 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling.
 Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

Mucicarmine

Product information

BenchMark Special Stains - Mucicarmine procedure

The Mucicarmine Staining Kit is intended for use as a qualitative histologic stain to detect mucopolysaccharides (mucin) in formalin fixed, paraffin-embedded tissue.

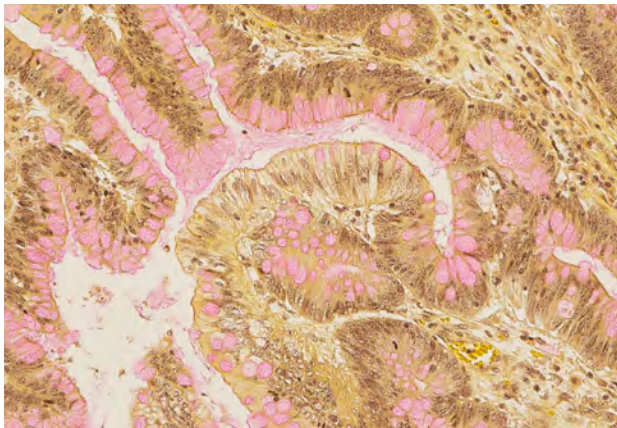


Figure 1. Colon adenocarcinoma stained with Mucicarmine stain, 200x.

Mucicarmine Staining Kit

Catalog number: 860-011

Ordering code: [05279275001](#)

Kit components

1. Mucicarmine stain
2. Iron Hematoxylin A contains 95% ethanol and 1% hematoxylin reagent
3. Iron Hematoxylin B contains 1.2% ferric chloride and 1% hydrochloric acid reagent
4. Tartrazine Counterstain contains <1% each of tartrazine and acetic acid reagent

Mucicarmine

Staining procedures protocol options

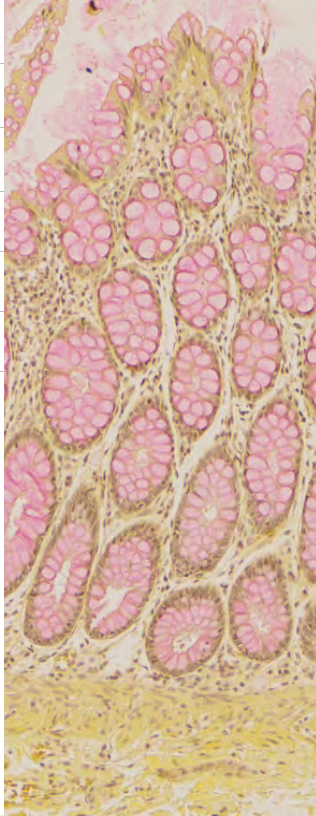
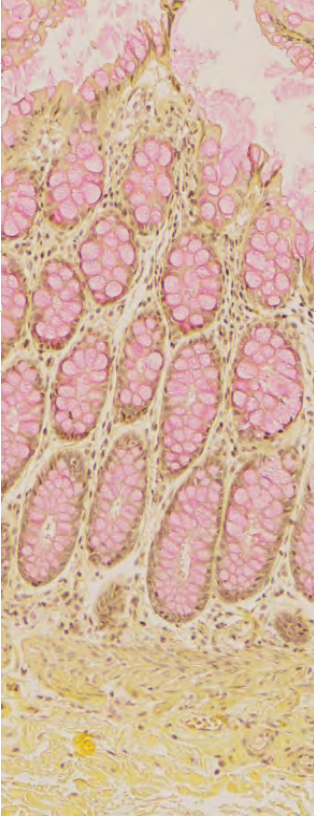
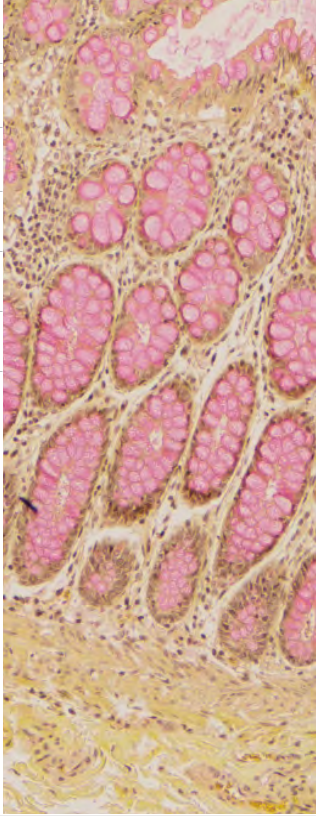
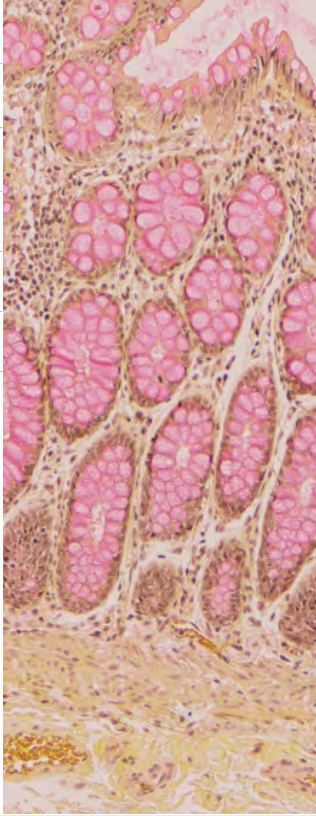
Mucicarmine	Shortest incubation times protocol	Time	Default protocol	Time	Alternative incubation times protocol	Time	Longest incubation times protocol	Time
Hematoxylin incubation: 8-16 min		8 min		8 min		12 min		16 min
Mucicarmine incubation: 8-16 min		8 min		12 min		16 min		16 min
Tartrazine incubation: 4-16 min		8 min		4 min		4 min		16 min

Figure 12- Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling.
Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

PAS

Product information

BenchMark Special Stains - PAS Staining procedure

The PAS Staining Kit uses Periodic Acid reagent to oxidize glycols to aldehydes.¹ The Schiff's Reagent forms a colorless dialdehyde compound that is transformed to the colored staining of glycol containing cellular components. PAS staining in tissue sections and digestion with a diastase reagent (860-004 /05279208001) is useful as an aid in the diagnosis of glycogen storage.²

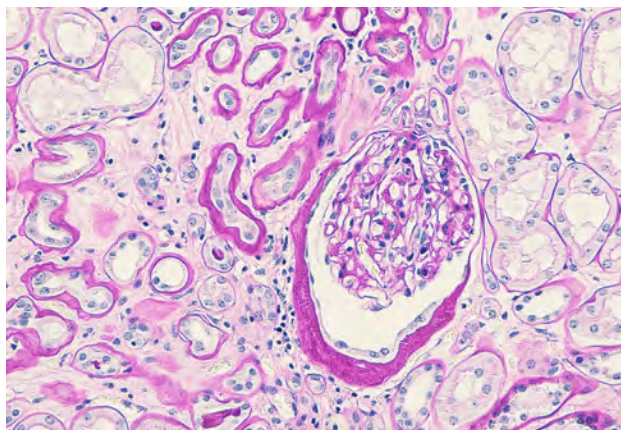


Figure 1. Kidney showing glomerular basement membrane stained with PAS stain, 200x.

PAS Staining Kit

Catalog number: 860-014

Ordering code: [05279291001](#)

Kit components

1. Periodic Acid contains less than 1% periodic acid
2. Schiff's Reagent contains 4% sodium bisulfite, 2% dilute hydrochloric acid and 1% pararosaniline chloride. (Three vials provided in the kit)
3. Neutralizer contains less than 1% sodium bisulfite
4. Hematoxylin Counterstain contains modified Mayer's hematoxylin

Light Green for PAS Staining Kit

Catalog number: 860-010

Ordering code: [05279267001](#)

Kit components

1. Light Green for PAS contains <1% light green SF yellowish in a 1% acetic acid solution

Diastase Kit

Catalog number: 860-004

Ordering code: [05279208001](#)

Kit components

1. Diastase contains 1.2% diastase of malt, with 0.1% sodium azide as a preservative

Alcian Blue for PAS Staining Kit

Catalog number: 860-003

Ordering code: [05279194001](#)

Kit components

1. Light Green for PAS contains <1% light green SF yellowish in a 1% acetic acid solution

References

1. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009
2. Thompson SW. Selected Histochemical and Histopathological Methods. Springfield; CC Thomas; 1966

PAS

Staining procedures protocol options

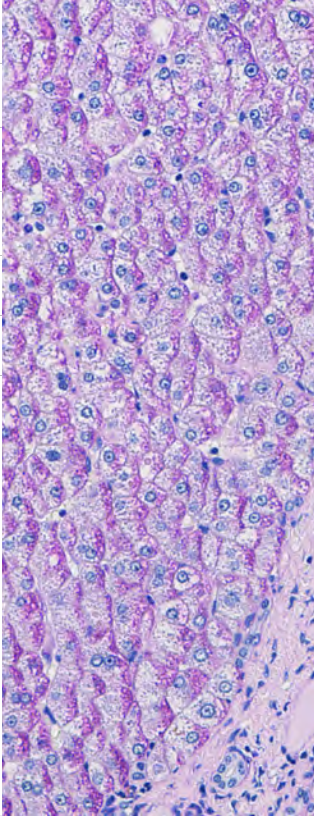
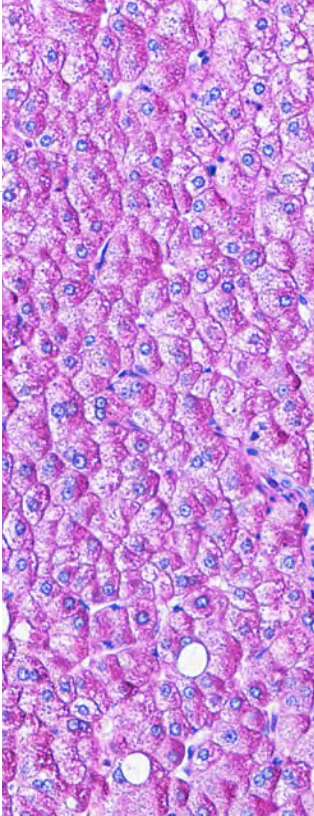
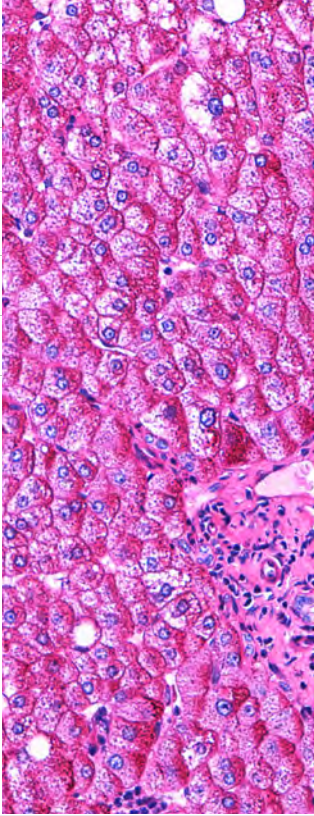
PAS	Shortest incubation time protocol	Time / temp	Default protocol	Time / temp	Longest incubation times protocol	Time / temp
Reaction temperature: 37-60°C		37°C		45°C		60°C
Schiffs: 12-20 min		12 min		20 min		20 min
Hematoxylin: 4-12 min		4 min		8 min		8 min
¹ Schiff's Reagent is stable for one month after opening. It is recommended that the vial be labeled with the date when opened for the first time. Sealed, unopened Schiff's Reagent is stable until the expiration date printed on the vial label. The kit contains two extra bottles of Schiff's Reagent to allow full use of the kit. As the open vial of Schiff reagent becomes unstable, higher background and less distinct staining of target components may be observed.						

Figure 2 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

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Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

PAS / Diastase

Staining procedures protocol options

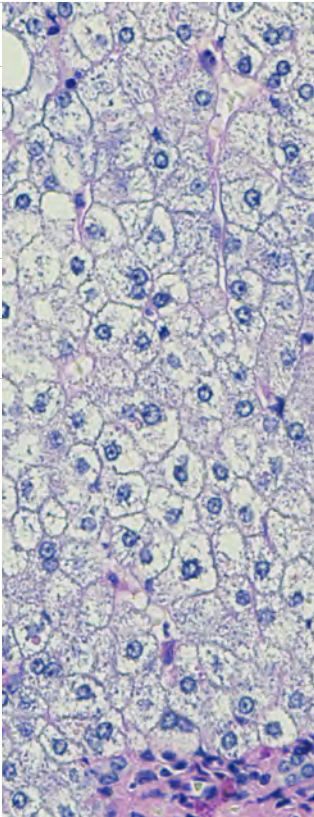
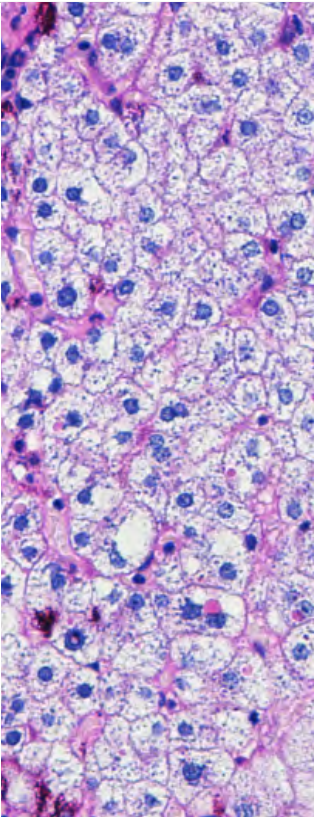
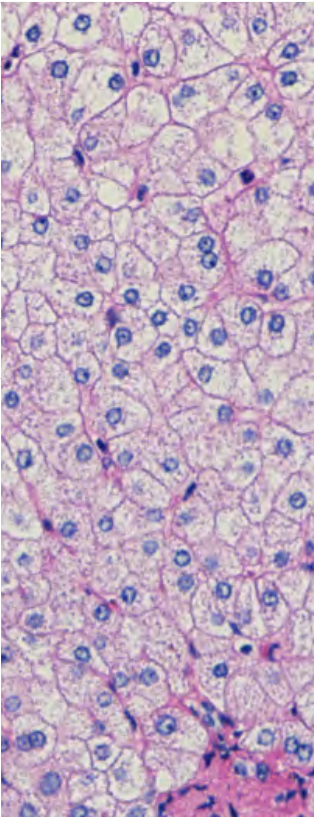
PAS / Diastase	Shortest incubation time (and optimized) protocol	Time / temp	Default protocol	Time / temp	Longest incubation times protocol	Time / temp
Reaction temperature: 37-60°C		37°C		50°C		60°C
Diastase: 4-16 min		4 min		4 min		16 min
Schiffs: 12-20 min		12 min		20 min		20 min
Hematoxylin: 4-12 min		4 min		8 min		12 min

Figure 3 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling.
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PAS / Light Green

Staining procedures protocol options

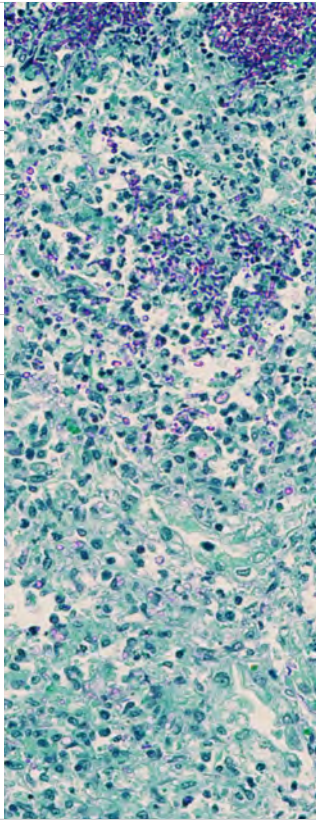
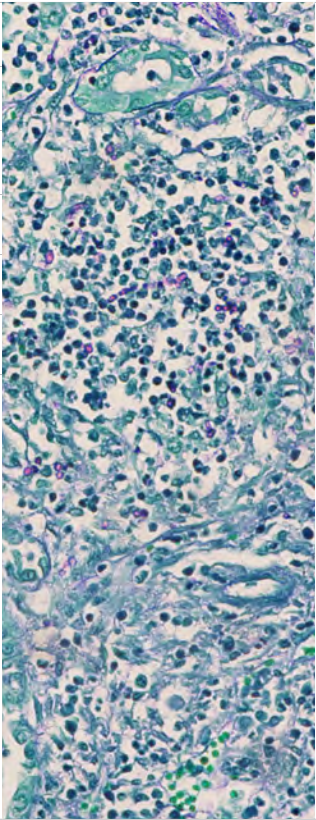
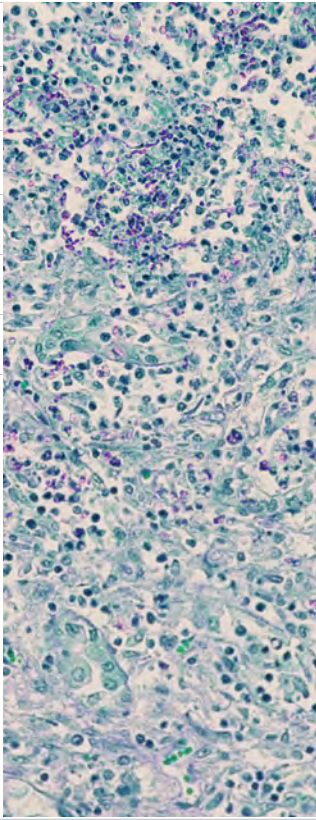
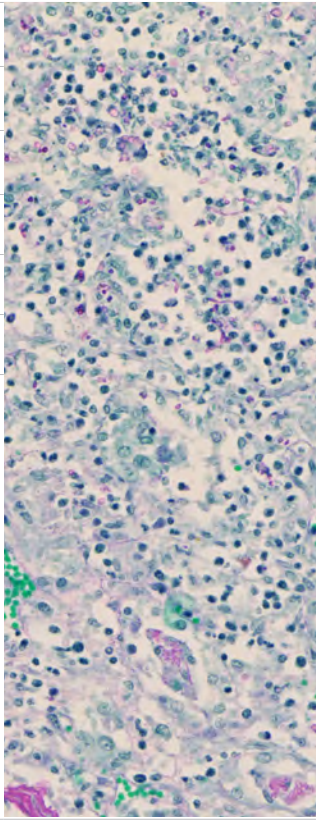
Light Green for PAS	Shortest incubation times protocol	Time / temp	Default protocol	Time / temp	Alternative incubation times protocol	Time / temp	Longest incubation times protocol	Time / temp
Reaction temperature: 37-60°C		37°C		45°C		37°C		60°C
Schiffs incubation: 12-20 min		12 min		20 min		20 min		12 min

Figure 4 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling.
Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

PAS / Alcian Blue

Staining procedures protocol options

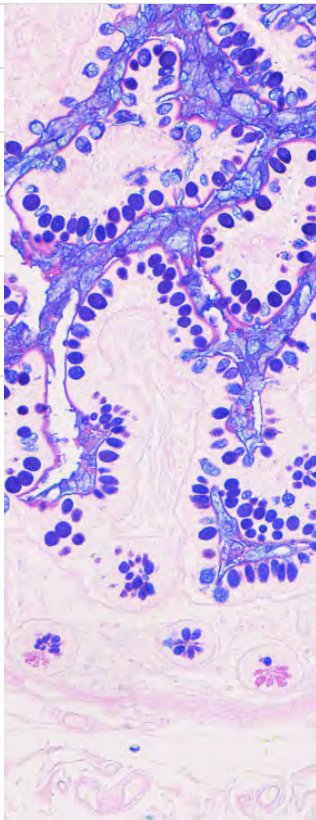
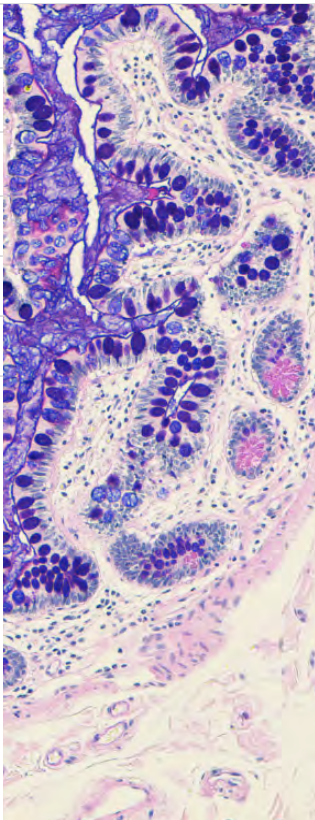
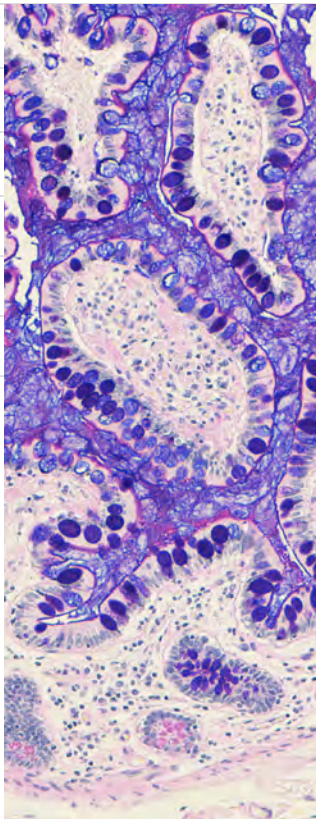
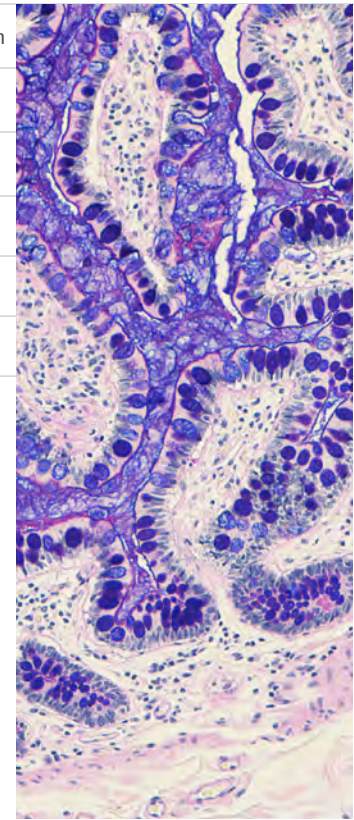
Alcian Blue for PAS	Shortest incubation times protocol	Time	Default incubation times protocol	Time	Alternative incubation times protocol	Time	Longest incubation times protocol	Time
Alcian Blue: 8-16 min		8 min		8 min		16 min		16 min
Hematoxylin: 4-12 min		0 min		4 min		4 min		12 min

Figure 5 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling. Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

Reticulum

Product information

BenchMark Special Stains - Reticulum II procedure

The Reticulum II Staining Kit is a modification of Gordon and Sweets Stain.¹ Oxidizer, with potassium permanganate, oxidizes the tissue to enhance staining of reticular fibers. Decolorizer, with oxalic acid, removes excess potassium permanganate. Sensitizer, with ferric ammonium sulfate, is added to form a metal organic compound. The metal organic compound is replaced by the silver in Reticulum II Silver A. Reducer is applied to develop the deposited silver into visible silver. Toner reagent contains gold chloride for better contrast and clarity. Fixer, with thiosulfate, stops the reaction and removes any unreacted silver from the section. Nuclear Fast Red Counterstain is applied to provide contrasting background.

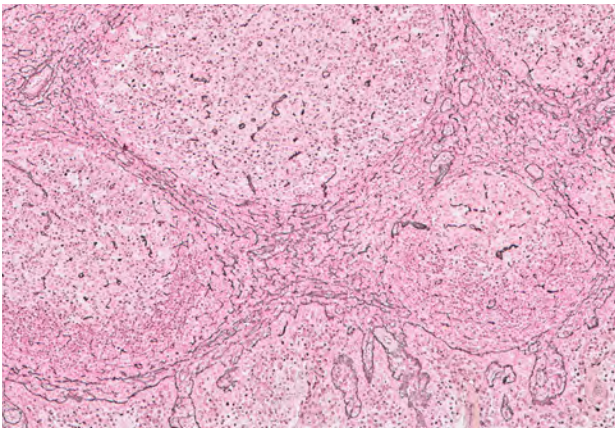


Figure 1. Tonsil stained with Retic stain, 400x.

Reticulum II Staining Kit

Catalog number: 860-024

Ordering code: [05729399001](#)

Kit components

1. Oxidizer contains less than 1% potassium permanganate
2. Decolorizer contains less than 1% oxalic acid
3. Sensitizer contains 2% ferric ammonium sulfate
4. Reticulum II Silver A contains less than 1.5% silver carbonate
5. Reticulum II Reducer contains 0.4% formaldehyde
6. Toner contains less than 1% gold chloride
7. Fixer II contains 2% sodium thiosulfate
8. Nuclear Fast Red Counterstain contains less than 1% Nuclear Fast Red and 5.0% aluminum sulfate

References

1. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009

Reticulum

Staining procedures protocol options

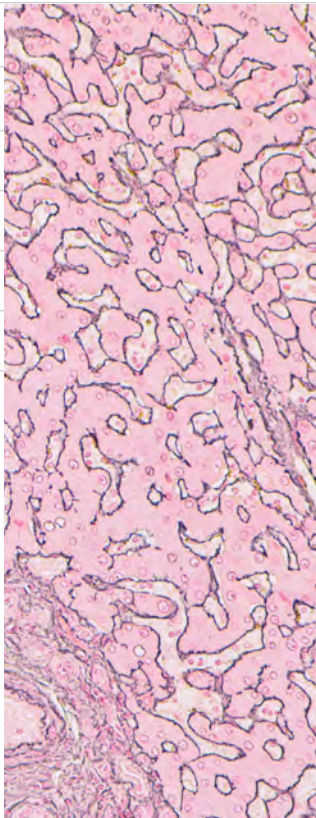
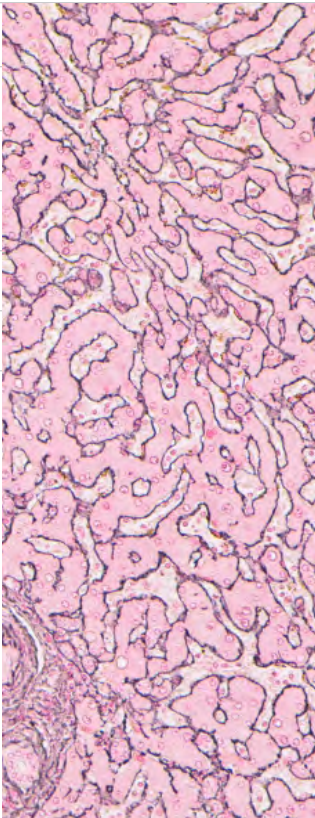
Reticulum	Shortest incubation times (and default) protocol	Time	Longest incubation times protocol	Time
Silver A: 8-16 min		8 min		16 min
NFR counterstain: 4-16 min		4 min		4 min

Figure 2 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling.
Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

Steiner II

Product information

BenchMark Special Stains - Steiner II procedure

The Steiner II Staining Kit is a silver-based kit used in diagnosis of many microorganisms, such as *Helicobacter pylori*, spirochetes, or *Legionella pneumophila*. These techniques have been reviewed by Garvey.¹

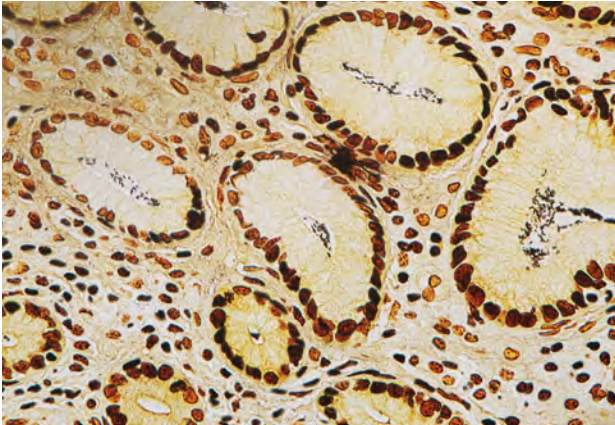


Figure 1. Gastric Biopsy positive for *H. Pylori*, 400x.

Steiner II Staining Kit

Catalog number: 860-030

Ordering code: 06521894001

Kit components

1. Steiner II Oxidizer contains 0.01% zinc chloride, 10% formalin
2. Steiner II Silver A contains 0.25% silver nitrate
3. Steiner II Fixer contains 1.0% sodium thiosulfate
4. Steiner II Diffuser contains 50% reagent alcohol
5. Steiner II Enhancer contains 5% gum mastic and absolute ethanol
6. Steiner II Clean A contains 95% reagent alcohol
7. Steiner II Reducer contains 0.8% hydroquinone
8. Steiner II Silver B contains 0.20% silver nitrate
9. Steiner II Clean B contains 95% reagent alcohol

References

1. Garvey W. Silver Impregnation Techniques to Identify Spirochetes and Other Bacteria. *J Histotechnol.* 1996;19(3):203-209

Steiner II

Staining procedures protocol options

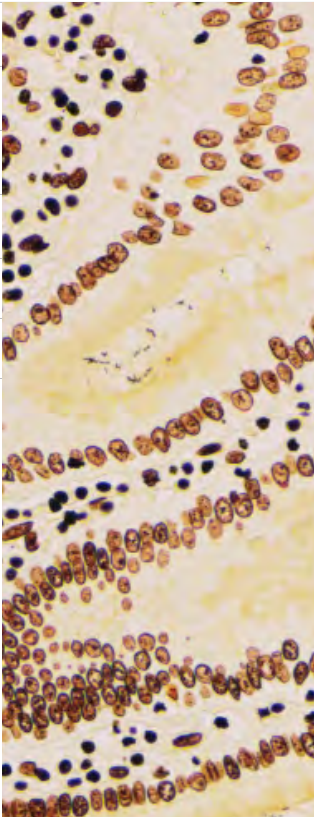
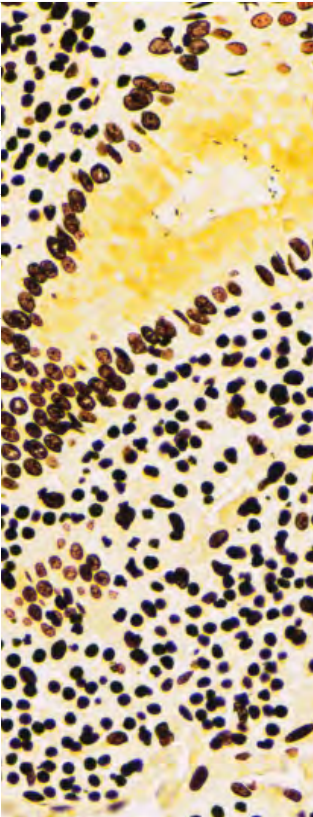
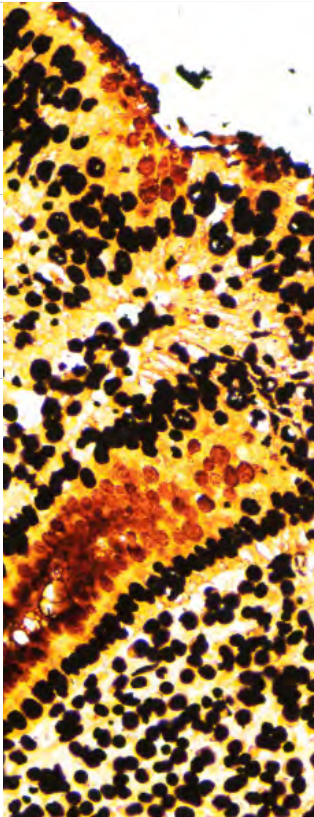
Steiner II	Shortest incubation times protocol	Time / temp	Intermediate incubation times protocol	Time / temp	Longest incubation times protocol	Time / temp
Reaction temperature: 50-65°C		50°C		52°C		65°C
Silver: 8-16 min		8 min		12 min		16 min

Figure 2 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling. Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

Trichrome Blue

Product information

BenchMark Special Stains - Trichrome Blue procedure

The Trichrome Staining Kit is a modification of Masson's trichrome stain. Bouin's solution is applied to tissue sections to intensify the final coloration. Cytoplasm and muscle are stained with Trichrome Red, containing Biebrich Scarlet and acid fuchsin. Nuclei are stained with iron hematoxylin. After application of Trichrome Mordant, the collagen is stained with Trichrome Blue, which contains aniline blue. Trichrome Clarifier, an acetic acid solution, is applied to create a more delicate and transparent shade of color in the tissue section.

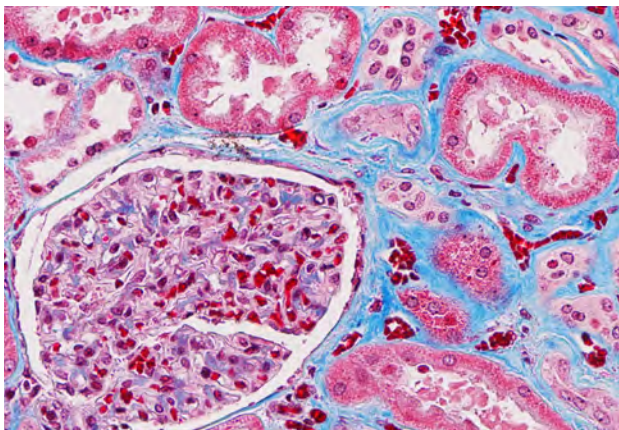


Figure 1. Kidney stained with Trichrome Blue, 200x.

Trichrome Staining Kit

Catalog number: 860-031

Ordering code: 06521908001

Kit components

1. Trichrome Bouin's reagent: A formulation of Bouin's fixative containing formaldehyde, acetic acid and picric acid. Since formalin fixation is not the best fixative when staining with acid dyes, Bouin's fixative is used in a secondary fixation step to correct the pH of the tissue and enable brighter dye staining
2. Trichrome Hematoxylin A and Trichrome Hematoxylin B: Trichrome Hematoxylin A and Trichrome B are two components of the nuclear stain. Trichrome Hematoxylin A is a hematoxylin reagent and Trichrome Hematoxylin B is a ferric chloride reagent. Together they form a complex of iron hematoxylin which stains the nuclei black
3. Trichrome Red: A formulation of the acid dyes Biebrich Scarlet and Acid Fuchsin which stains the red blood cells, muscle and cytoplasmic tissue components
4. Trichrome Mordant: A formulation of the metal salts of phosphotungstic acid and phosphomolybdic acid. It serves as a differentiating agent, removing the excess red from the collagen which is subsequently stained blue with Trichrome Blue
5. Trichrome Blue: A formulation of Aniline Blue used to stain the collagen tissue components
6. Trichrome Clarifier: An acetic acid solution used as a final rinse to remove excess blue and to refine the staining

Trichrome Blue

Staining procedures protocol options

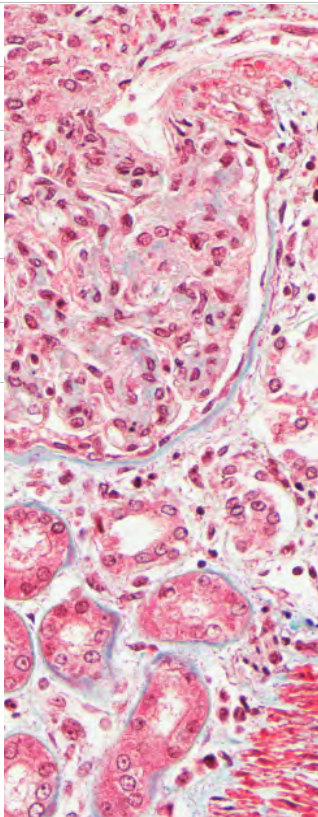
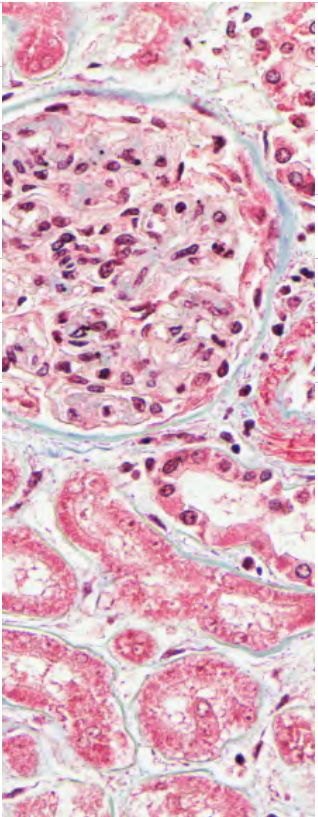
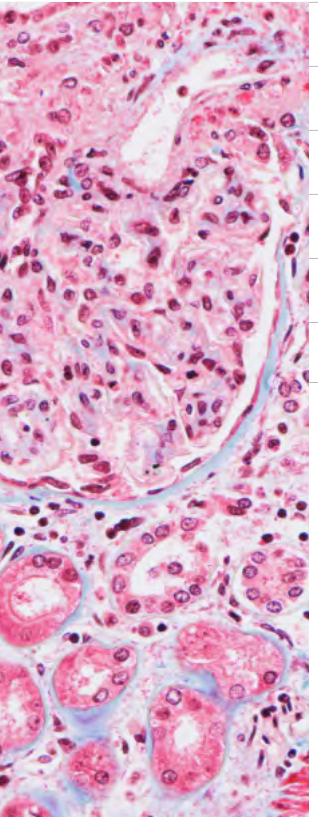
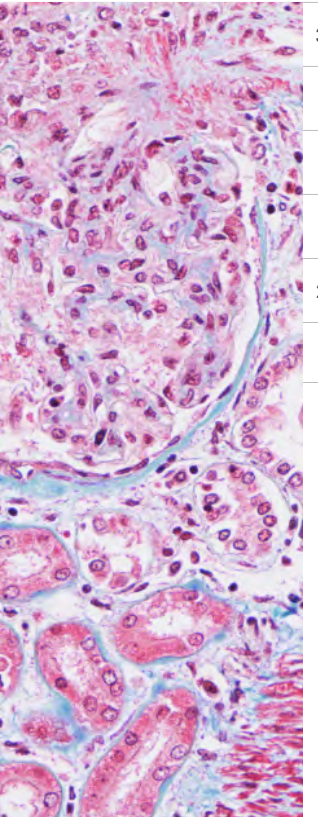
Trichrome Blue	Shortest incubation times protocol (kidney)	Time	Default incubation times protocol	Time	Darkest incubation times protocol (kidney)	Time	Optimized protocol for kidney and liver biopsies	Time
Bouins: 32-64 min		32 min		32 min		32 min		32 min
Hematoxylin time: 4-24 min		4 min		12 min		24 min		12 min
Trichrome Red time: 4-24 min		4 min		12 min		24 min		12 min
Mordant: 4-24 min		4 min		12 min		24 min		12 min
Blue: 4-24 min		4 min		4 min		24 min		24 min

Figure 2 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

This field guide is intended to be an educational supplement, not a substitute for product labeling.
Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

Trichrome Blue

Staining procedures protocol options

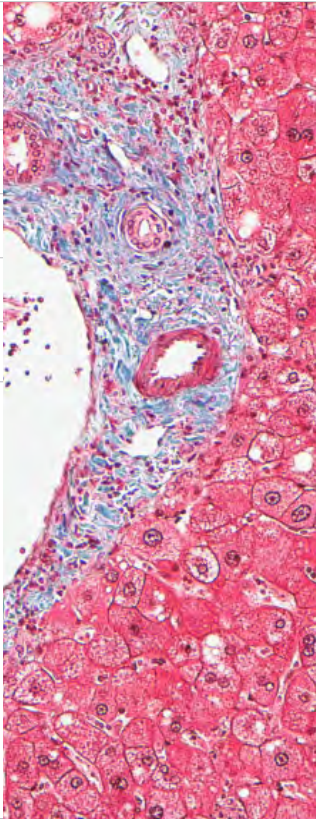
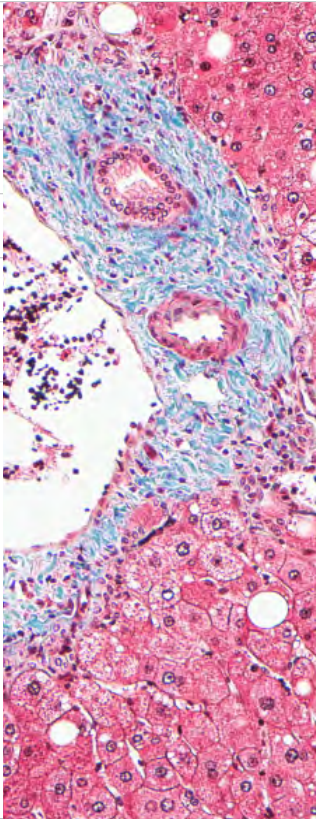
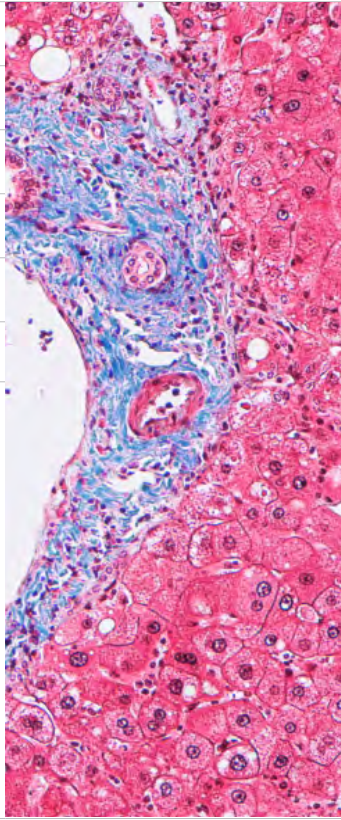
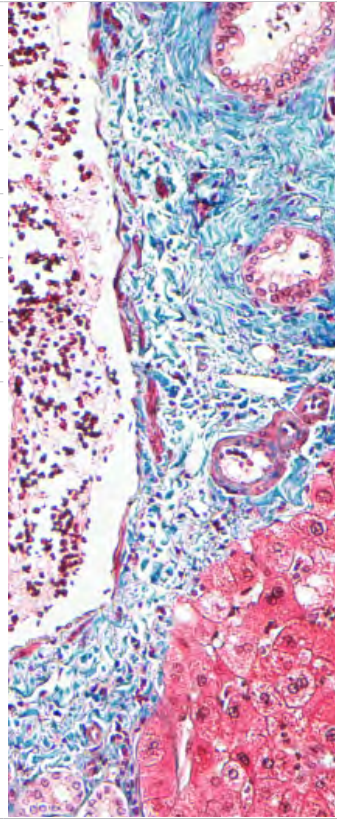
Trichrome Blue	Shortest incubation times protocol (kidney)	Time	Default incubation times protocol	Time	Darkest incubation times protocol (kidney)	Time	Optimized protocol for kidney and liver biopsies	Time
Bouins: 32-64 min		32 min		32 min		32 min		32 min
Hematoxylin time: 4-24 min		4 min		12 min		24 min		12 min
Trichrome Red time: 4-24 min		4 min		12 min		24 min		12 min
Mordant: 4-24 min		4 min		12 min		24 min		12 min
Blue: 4-24 min		4 min		4 min		24 min		24 min

Figure 3 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

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Trichrome Green

Product information

BenchMark Special Stains – Trichrome Green procedure

Green for Trichrome is a single bottle kit, which is used in conjunction with the Trichrome Staining Kit. The Trichrome Staining Kit is a modification of Masson's Trichrome Stain. Trichrome Bouin's is applied which acts as a mordant to allow penetration of subsequent dyes. Nuclei are stained with Trichrome Hematoxylin A and Trichrome Hematoxylin B (forms a complex of iron hematoxylin). Cytoplasm and muscle is stained with Trichrome Red, containing Biebrich scarlet and acid fuchsin. Trichrome Mordant removes the excess red from the collagen which is stained with Green for Trichrome, which contains fast green. Trichrome Clarifier is an acetic acid rinse used to remove excess green. This kit is optimized for use on the BenchMark Special Stains automated slide stainers. The reagents are applied to tissue on microscope slides and mixed over the entire specimen. The staining reaction is based on the differential effect of acid dye on muscle and collagen.

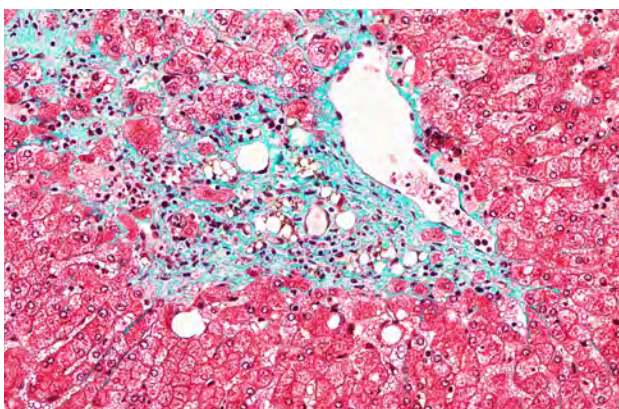


Figure 1. Liver stained with Trichrome Green, 200x.

Trichrome Staining Kit

Catalog number: 860-031

Ordering code: 06521908001

Kit components

1. Trichrome Bouin's reagent: A formulation of Bouin's fixative containing formaldehyde, acetic acid and picric acid. Since formalin fixation is not the best fixative to use when staining with acid dyes, Bouin's fixative is used in a secondary fixation step to correct the pH of the tissue and enable brighter dye staining
2. Trichrome Hematoxylin A and Trichrome Hematoxylin B: Trichrome Hematoxylin A and Trichrome B are two components of the nuclear stain. Trichrome Hematoxylin A is a hematoxylin reagent and Trichrome Hematoxylin B is a ferric chloride reagent. Together they form a complex of iron hematoxylin which stains the nuclei black
3. Trichrome Red: A formulation of the acid dyes Biebrich Scarlet and Acid Fuchsin which stains the red blood cells, muscle and cytoplasmic tissue components
4. Trichrome Mordant: A formulation of the metal salts of phosphotungstic acid and phosphomolybdic acid. It serves as a differentiating agent, removing the excess red from the collagen which is subsequently stained green with Fast Green
5. Trichrome Blue: This is not used in Trichrome Green B procedure
6. Trichrome Clarifier: An acetic acid solution used as a final rinse to remove excess green and to refine the staining

Green for Trichrome Staining Kit

Catalog number: 860-032

Ordering code: 06521916001

Kit components

1. Trichrome Green reagent: A Fast Green and weak hydrochloric acid solution

Trichrome Green

Staining procedures protocol options

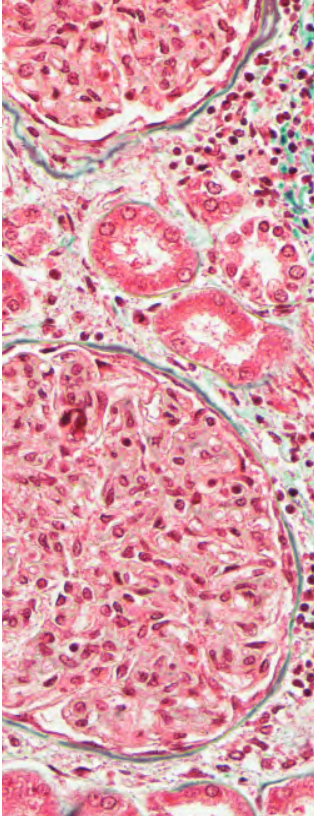
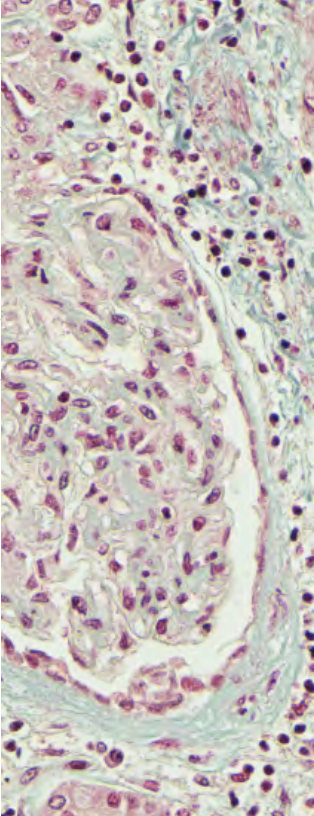
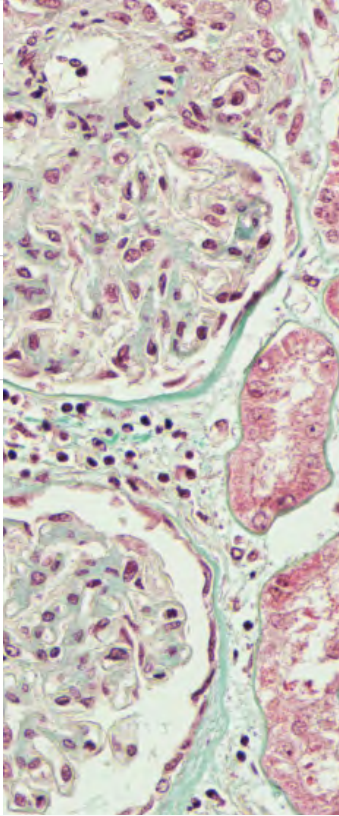
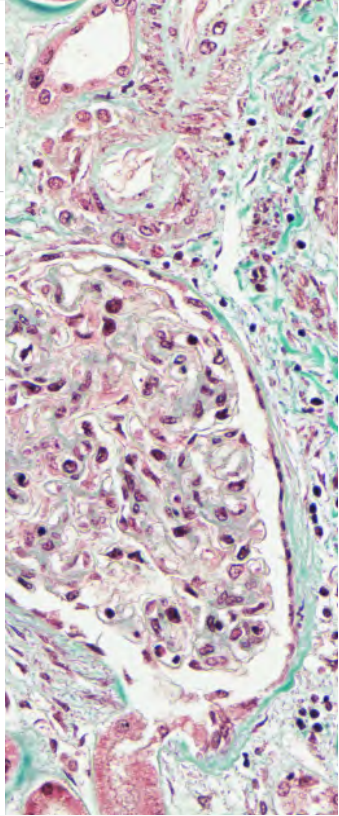
Trichrome Green	Shortest incubation times protocol (kidney)	Time	Default incubation times protocol (kidney)	Time	Longest incubation times protocol (kidney)	Time	Optimized protocol for kidney and liver biopsies	Time
Bouins: 32-64 min		32 min		32 min		32 min		32 min
Hematoxylin time: 4-24 min		4 min		4 min		24 min		12 min
Trichrome Red time: 4-24 min		4 min		4 min		24 min		12 min
Mordant: 4-24 min		4 min		4 min		24 min		12 min
Green: 4-32 min		4 min		4 min		24 min		12 min

Figure 2 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

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Trichrome Green

Staining procedures protocol options

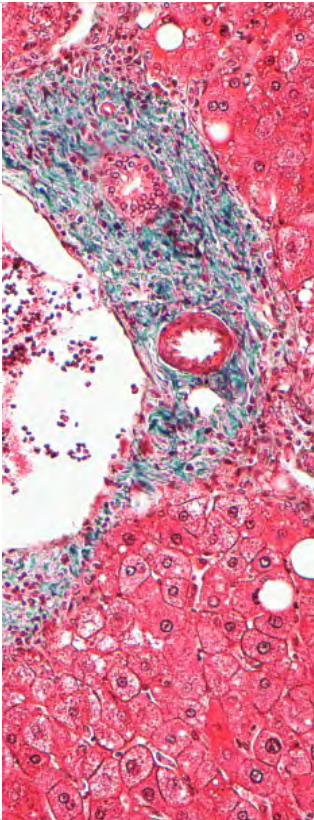
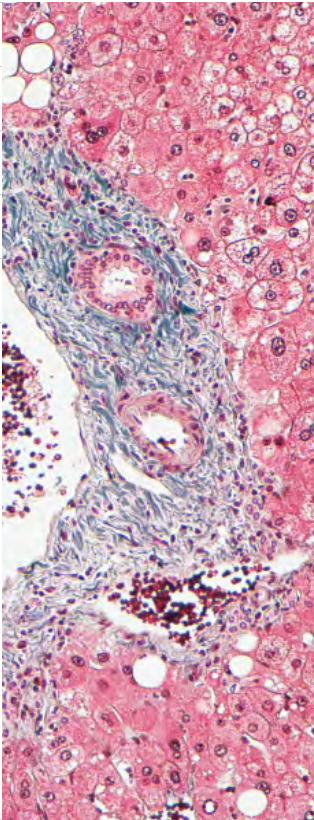
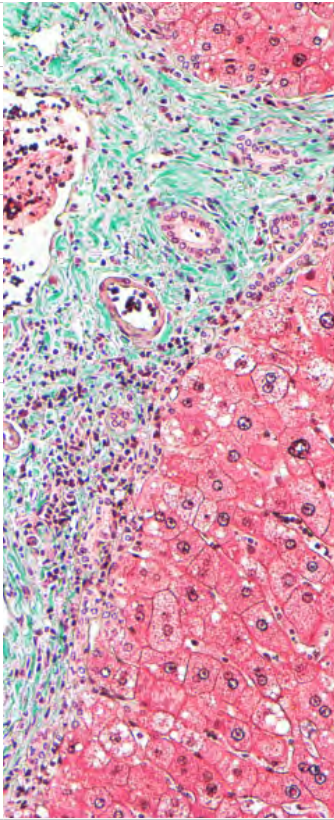
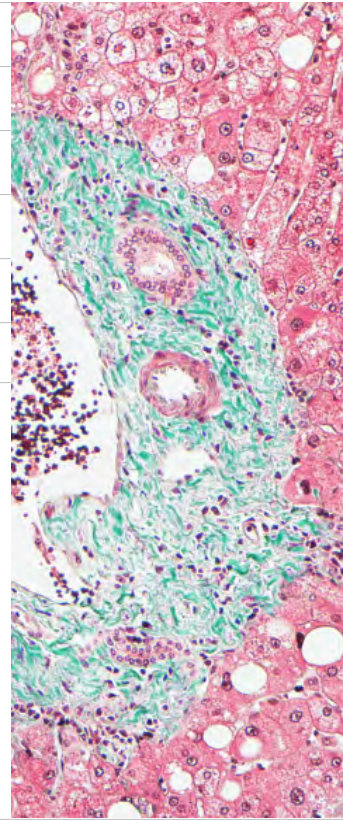
Trichrome Green	Shortest incubation times protocol (liver)	Time	Default incubation times protocol (liver)	Time	Longest incubation times protocol (liver)	Time	Optimized protocol (for kidney and liver biopsies) (liver)	Time
Bouins: 32-64 min		32 min		32 min		32 min		32 min
Hematoxylin time: 4-24 min		4 min		12 min		24 min		12 min
Trichrome Red time: 4-24 min		4 min		12 min		24 min		12 min
Mordant: 4-24 min		4 min		12 min		24 min		12 min
Green: 4-24 min		4 min		4 min		24 min		24 min

Figure 3 - Sample images of staining achieved with different protocol time selections.

* Key to staining procedures protocol located on page 3

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Refer to the package insert and operator manual for primary information regarding your special stains kits and instrument operation.

General technical notes and post-instrument processing

Technical notes

1. Known positive tissue controls should be utilized for monitoring the correct performance of processed tissues and test reagents. Control tissue should be fresh autopsy, biopsy or surgical specimen prepared or fixed as soon as possible in a manner identical to test sections. Such tissues should monitor all steps of the analysis, from tissue preparation through staining. Use of a tissue section fixed or processed differently from the test specimen provides control for all reagents and method steps except fixation and tissue processing. The cellular components of other tissue elements may serve as the negative control.
2. You can either bake the slides on the BenchMark Special Stains instrument or use an alternative method off the instrument. Be aware that, with some paraffins, baking at excessively high temperatures may adversely affect the staining. Check the temperature recommendations for the paraffin used in your lab.
3. Necrotic or autolyzed tissue may exhibit nonspecific staining.
4. At the end of the run there will be residual Liquid Coverslip left on the slide. This will be removed through dehydration with alcohol and xylene.

Do not rinse slides in water and DAWN® detergent.

Kit storage information

The secondary packaging (box) on each of the special stains kits indicates the appropriate storage conditions for the entire contents of the kit. Individual vials are labeled with specific storage conditions that may include a broader range than indicated on the secondary packaging. Individual reagent vials with a gray collar that are part of a cold storage kit may be stored at room temp or cold storage (2-30°C). Individual reagent vials that must be stored at 2-8°C have a blue collar.

Components that are stored cold must be brought to room temperature prior to use to achieve optimal results.

Inappropriate storage may shorten the life of the product and cause unsatisfactory results.

Refer to the package insert for additional guidance on storage conditions.

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