Green for Trichrome

REF 860-032

06521916001

IVD Σ 75

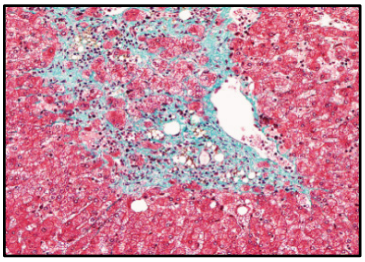

Figure 1. Green for Trichrome staining liver tissue.

INTENDED USE

Green for Trichrome, in conjunction with Trichrome Staining Kit, is intended for laboratory use as a qualitative histologic stain to demonstrate collagen fibers, muscle and connective tissue by light microscopy in sections of formalin-fixed, paraffin-embedded (FFPE) tissue stained on a BenchMark Special Stains instrument.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This product is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

Green for Trichrome is a single bottle kit which is used in conjunction with the Trichrome Staining Kit. Trichrome Staining Kit is a modification of Masson's Trichrome Stain.[1]

Fibrosis occurs when normal tissue architecture and function are compromised due to the presence of excess extracellular matrix components.[2] Fibrosis can occur in several tissues/organs and can lead to permanent scarring and organ malfunction.[3] The primary marker of fibrosis is overgrowth and/or scarring which is associated with collagen volume.[4] The trichrome staining method employs three dyes that distinctly color smooth muscle, collagen, and connective tissues.[5] This staining allows for a more accurate assessment of collagen in a tissue specimen by enhancing contrast of these tissue components.

Green for Trichrome, in conjunction with the Trichrome Staining Kit, is used to aid the pathologist in the assessment of fibrosis.[6]

PRINCIPLE OF THE PROCEDURE

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.

This kit is optimized for use on BenchMark Special Stains instruments. The reagents are applied to tissue on microscope slides and mixed over the entire specimen.

MATERIAL PROVIDED

The reagent vials are supplied in barcode labeled carriers to insert into the reagent tray of the instrument. Each kit contains sufficient reagent for 75 tests:

One 27 mL vial of Trichrome Green reagent contains approximately 1% fast green and 0.75% hydrochloric acid.

One vial insert with sipping straw.

Reconstitution, Mixing, Dilution, Titration

No reconstitution, mixing, dilution, or titration of kit reagent is required. Further dilution of the reagent may result in unsatisfactory staining.

The reagent in this kit have been optimally diluted for use on BenchMark Special Stains instruments.

MATERIALS REQUIRED BUT NOT PROVIDED

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. BenchMark Special Stains instrument
4. BenchMark Special Stains Deparaffinization Solution (10X) (Cat. No. 860-036 / 06523102001)
5. BenchMark Special Stains Liquid Coverslip (Cat. No. 860-034 / 06523072001)
6. BenchMark Special Stains Wash II (Cat. No. 860-041 / 08309817001)
7. Trichrome Staining Kit (Cat. No. 860-031 / 06521908001)
8. General purpose laboratory equipment

STORAGE AND STABILITY

The Green for Trichrome should be stored at 2-8°C. Refrigerated kit components should be brought to room temperature prior to use.

When properly stored, unopened and opened reagents are stable until the expiration date that is printed on the vial label.

Do not use reagent beyond the expiration date indicated on the kit.

There are no obvious signs to indicate instability of these reagents; therefore, controls should be run simultaneously with unknown specimens. Contact your local support representative if positive control material shows a decrease in staining as it could indicate reagent instability.

SPECIMEN PREPARATION

Routinely processed, FFPE tissues are required for use with this product and BenchMark Special Stains instruments. The recommended tissue fixative is 10% neutral buffered formalin.[7]

Perform specimen collection and storage according to CLSI document M29-T2.[8] Cut sections to the appropriate thickness, approximately 2-4 µm, and place the sections on positively charged glass slides.

1. Dry the slides.[7]
2. Print appropriate barcode label(s).
3. Apply barcode labels to the frosted end of the slides prior to loading the slides onto the instrument (see the instrument User Guide for correct application of labels).

Refer to the Instructions for Use section for the recommended protocol for the BenchMark Special Stains instrument.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. **CAUTION**: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[9,10]
7. Avoid contact with eyes and mucous membranes. If reagent contacts these areas, rinse with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results
9. For further information on the use of this device, refer to the BenchMark Special Stains instrument User Guide, and instructions for use of all necessary components located at dialog.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.

12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Warning 	H290	May be corrosive to metals.
	P234	Keep only in original packaging.
	P390	Absorb spillage to prevent material damage.

INSTRUCTIONS FOR USE

Prepare Reagent Vial

Before first use, a vial insert and sipping straw must be placed in the reagent vial.

Remove the shipping cap from the vial and place the insert and straw into the vial. The insert and sipping straw should be left in the vial, once the vial has been opened.

Staining Procedure

1. Load reagents and slides onto the instrument.
2. Place the soft cap into the slot on the reagent holder when the reagent is in use.
3. Perform the staining run according to the recommended protocols in Table 2 and the instructions in the User Guide.
4. When the run is complete, remove the slides from the instrument.
5. Use the soft cap to cover the reagent vial when reagent is not in use.
6. After use, store the reagents according to the recommended storage conditions.

Recommended Protocol

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide.

The following procedures allow flexibility to accommodate user preference. This product has been optimized for use with the BenchMark Special Stains instrument but the user must validate results obtained with this product.

Table 2. Recommended staining protocol for Green for Trichrome in conjunction with Trichrome Staining Kit on a BenchMark Special Stains instrument.

Staining Procedure	S Trichrome
Protocol Step	**Method**
Deparaffinization	Select to automate paraffin removal.
Baking (optional)	The default is not selected. 75°C for 8 minutes is recommended.
Bouins	Bouins step is recommended to further tissue fixation to provide a brighter dye staining. Select Bouins to enable the default time of 32 minutes. Select Optimize Bouins to enable adjustment.* Select an incubation time from 4 to 32 minutes. ~2 µm tissue sections: Bouins default is recommended.
Extended Bouins (Optional)	Select to enable Extended Bouins incubation. Time will be added to default Bouins incubation time (32 minutes). Select an incubation time from 4-32 minutes.* ~4 µm tissue sections: 28 minutes of extended incubation is recommended.
Blue or Green	Select Green to run the default protocol.
Optimize Hematoxylin Intensity (Hematoxylin A and B)	The default is Option 1 for 16 minutes. Select to enable Option selection.* Option 1 enables dispense order of Hematoxylin A then B. Option 2 may be used for brighter red staining and enables dispense order of Hematoxylin B then A. Select enable adjustment of incubation time:* 4 minutes, lighter staining of nuclei 24 minutes, darker staining of nuclei ~2 µm tissue sections: 4 minutes is recommended.
Optimize Red Intensity for Green (Trichrome Red)	The default is 37°C for 16 minutes. Select to enable adjustment of staining intensity:* 37-60°C incubation temperature 4 to 24 minute incubation time ~2 µm tissue sections: 60°C for 24 minutes is recommended.
Optimize Mordant for Green (Trichrome Mordant)	The default is both Mordant 1 and Mordant 2 dispenses for 4 minutes each. The default temperature is 37°C for Mordant 1 and 40°C for Mordant 2. Select to enable adjustment of Mordant 1 and/or 2 incubation time and temperature:* 37-60°C incubation temperature 4 to 24 minute incubation time Select No 1st or 2nd Mordant dispense to skip the dispense of Mordant 1 or 2 (only one can be selected). ~2 µm tissue sections: Mordant 1 only at 50°C for 4 minutes is recommended.
Optimize Green Intensity (Trichrome Green)	The default is No Heat for 24 minutes. Select to enable the adjustment of staining intensity.* Select an incubation temperature and time: No Heat for Green 37-60°C incubation temperature 4 to 32 minute incubation ~2 µm tissue sections: 37°C for 24 minutes is recommended.

* To adjust staining preferences, increment the stain temperature and incubation time one parameter at a time.

Recommended Post-Instrument Processing

1. Rinse slides in two changes of 95% ethanol to remove the leftover solution, followed by three changes of 100% ethanol.
2. Dehydrate slides in three changes of 100% xylene.
3. Coverslip with permanent mounting media.

Compatible with the VENTANA HE 600 system coverslipping protocol. For further instructions, refer to the VENTANA HE 600 system User Guide.

QUALITY CONTROL PROCEDURE

An example of a positive control material would be FFPE human tissue of colon, kidney, liver or skin. 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.

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control.

The control tissue must be tested with each run.

Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, not as an aid in formulating a specific diagnosis of patient samples.

If the positive tissue components fail to demonstrate positive staining, results with the test specimens should be considered invalid. If the negative components demonstrate positive staining, results with patient specimens should also be considered invalid.

Unexplained discrepancies in control results should be referred to the local support representative immediately. If quality control results do not meet specifications, patient results are invalid. The cause must be identified and corrected, and the patient samples repeated.

STAINING INTERPRETATION / EXPECTED RESULTS

Green for Trichrome, used in conjunction with the Trichrome Staining Kit, is tested to demonstrate collagen fibers, muscle and connective tissue.

- Collagen Fibers: Green
- Muscle Fibers: Red
- Erythrocytes: Red to Red-Black
- Nuclei: Red to Red-Black

SPECIFIC LIMITATIONS

Only positively charged microscope slides have been used and validated for this assay.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Analytical sensitivity and specificity was evaluated in normal and diseased tissues cut at approximately 4 µm. All evaluated tissue cases (82/82) passed for acceptable staining as shown in Table 3 and 4.

Table 3. Sensitivity/Specificity of Green for Trichrome was determined by testing the following FFPE normal tissues.

Tissue	# cases passed / # tested
Kidney	5 / 5
Liver	5 / 5
Heart	5 / 5
Skin	5 / 5
Colon	5 / 5
Skeletal muscle	11 / 11

Table 4. Sensitivity/Specificity of Green for Trichrome was determined by testing the following FFPE diseased tissues.

Tissue	# cases passed / # tested
Focal segmental glomerulosclerosis (Kidney)	4 / 4
Glomerular disease (Kidney)	4 / 4
Membranous glomerulonephritis (Kidney)	3 / 3
Membranoproliferative glomerulonephritis (Kidney)	3 / 3
Lupus nephropathy (Kidney)	6 / 6
Diabetic glomerulosclerosis (Kidney)	3 / 3
Collagenous colitis (Colon)	11 / 11
Cirrhosis (Liver)	12 / 12

Precision

Precision of Green for Trichrome was determined across multiple runs, days, instruments, and reagent lots using multiple cut slides from 2 normal liver tissue cases, 2 normal kidney tissue cases and 2 liver (cirrhosis) tissue cases. Test cases were cut at approximately 4 µm. All acceptance criteria were fully met. Precision slides studies were performed for Green for Trichrome according to Table 5.

Table 5. Precision slide studies for Green for Trichrome.

Parameters Tested	# of conditions	# slides passed / # tested
Run to Run	3 runs, same day	54 / 54
Day to Day	5 days	90 / 90
Instrument to Instrument	3 instruments	54 / 54
Intra Run	same day, same instrument	54 / 54
Lot to lot	3 lots	54 / 54

The results demonstrated no significant difference in staining intensity among the slides.

Additional Testing

Additional testing was performed to demonstrate acceptable staining using tissue cases cut at approximately 2 µm. Staining procedure parameters were adjusted within the ranges specified in Table 2. All tested tissue types (2 normal liver tissue cases, 1 normal kidney tissue case, 2 kidney (glomerulopathy) tissue cases and 3 liver (cirrhosis)) fully met the acceptance criteria for acceptable staining.

TROUBLESHOOTING

1. Section thickness may affect quality and intensity of staining. If staining is inappropriate, contact your local support representative for assistance.
2. Necrotic or autolyzed tissue may exhibit nonspecific staining.
3. If the positive control is negative, tissue may have been improperly collected, fixed, or deparaffinized. Follow the proper procedure for collection, storage, and fixation.
4. If the positive control is negative, check that the slide has the proper barcode label. If the slide is labeled properly, check the other positive controls from the same run to determine if the controls were properly stained.
5. If excessive background staining occurs: incomplete paraffin removal could cause staining artifacts or no staining. If all paraffin is not removed from the slide, repeat the staining run using the extended deparaffinization option, if available.
6. If tissue sections wash off the slide, confirm the slides are positively charged.
7. Adjustment of one or multiple protocol parameters affects the staining intensity of all the tissue elements. For additional optimization guidance, contact your local support representative.
8. Extended stay of the slides on-board the instrument after run completion may affect quality and intensity of the staining. If the staining is inappropriate, remove slides promptly at the end of the run.
9. For corrective action, refer to the Instructions for Use section, the instrument User Guide or contact your local support representative.

REFERENCES

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8. Clinical and Laboratory Standards Institute (CLSI). CLSI Web site. http://www.clsi.org/. Accessed November 3, 2011.
9. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
H	Updates to Warnings and Precautions and Intellectual Property

INTELLECTUAL PROPERTY

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