Green for Trichrome

REF 860-032

06521916001

IVD Σ 75

INTENDED USE

Green for Trichrome with Trichrome Staining Kit (860-031 / 06521908001) is a qualitative histologic stain used to study connective tissue, muscle and collagen fibers in formalin-fixed, paraffin-embedded tissue.

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 stains are used to differentiate collagen from muscle tissue.[1] They are usually nuclear and cytoplasmic stains, followed by a mordant, typically phosphotungstic and phosphomolybdic acid, followed by a collagen fiber stain. The first Trichrome system has been attributed to Mallory.[2] Further modifications were introduced by Masson, Gomori, and by Lillie.[3] Trichrome stains are useful for indicating fibrotic change, that is, an increase in collagen like that which occurs in cirrhosis of the liver and pyelonephritis. Trichrome stains can be useful for distinguishing histologic changes that occur in neuromuscular diseases. They are also useful for differentiating tumors that originated in muscle cells from those that originated in fibroblasts.

PRINCIPLE OF THE PROCEDURE

VENTANA 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. This kit is optimized for use on VENTANA 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.

MATERIALS AND METHODS

Reagents Provided

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

One 27 mL vial of Trichrome Green reagent contains less than or equal to 1% fast green and 0.75% hydrochloric acid.

1 vial insert with sipping straw.

Reconstitution, Mixing, Dilution, Titration

No reconstitution, mixing, dilution, or titration of kit reagents is required. Further dilution of any of the reagents may result in unsatisfactory staining quality. The user must validate any such change.

The reagents in this kit have been optimally diluted for use on BenchMark Special Stains automated slide stainers These reagents may not be optimal for manual procedures or for use on other instruments.

Materials and Reagents Needed But Not Provided

1. Control tissue
2. Microscope slides (positively charged)
3. Drying oven capable of maintaining a temperature of 70°C ± 5°C
4. BenchMark Special Stains automated slide stainer and associated bulk reagents
5. Xylene or other clearing reagent (Histological grade)
6. Reagent alcohol or ethanol (Histological grade)
7. Deionized or distilled water
8. Synthetic mounting media
9. Coverslip
10. Trichrome Staining Kit (860-031)

Storage and Handling

The Green for Trichrome should be stored at 2-8°C. See vial label for proper storage conditions. Refrigerated kit components should be brought to room temperature prior to use.

When properly stored, reagents are stable until the expiration date that is printed on the vial label. There are no obvious signs to indicate instability of these reagents; therefore, controls should be run simultaneously with unknown specimens. If positive control material shows a decrease in staining it could indicate reagent instability and your local support representative should be contacted immediately.

Specimen Collection and Preparation for Analysis

Ventana recommends specimen collection and storage be performed according to CLSI document M29-T2.[4] The recommended tissue fixative is 10% neutral buffered formalin.[1]

1. Cut sections, usually 2 to 5 µm, and pick up the sections on glass slides.
2. You can either bake the slides on the BenchMark Special Stains instrument or use an alternative method off the instrument.
   - If you choose to bake the slides on the BenchMark Special Stains instrument, proceed to step 3.
   - Off the instrument, bake the slides for at least 30 minutes at approximately 70°C. Allow to cool.
3. Print appropriate barcode label(s).
4. Apply barcode labels to the frosted end of the slides prior to deparaffinization (see the instrument Operator's Manual for correct application of labels).
5. Refer to the Instructions for Use section for the recommended protocol for the BenchMark Special Stains automated slide stainer.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use.
2. For professional use only.
3. Consult local and/or state authorities with regard to recommended method of disposal.
4. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. Use universal precautions when handling and disposing of specimens.
5. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
6. These reagents may cause irritation. Avoid contact with eyes and mucous membranes. If reagent contacts these areas, rinse with copious amounts of water.
7. Trichrome Green may cause severe burns.
8. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Hazard Guide located at www.ventana.com.

INSTRUCTIONS FOR USE

Prepare Reagent Vial

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

1. 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.
2. Place the soft cap into the slot on the reagent holder when the reagent is in use.
3. Use the soft cap to cover the reagent vial when reagent is not in use.

Staining Procedure

1. Load reagents and slides onto the instrument.
2. Perform the staining run according to the recommended protocols (see Tables 1 and 2) and the instructions in the manual.
3. When the run is complete, remove the slides from the instrument.

Recommended Protocol

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument Operator's Manual.

The following procedures allow flexibility to accommodate varying tissue thickness, size and user preference. Trial runs using the protocols are suggested to adjust staining to the

user's preference. The recommended protocols are a starting point and adjustments may be necessary to match individual user's preference, pre-analytics and needs.

The following protocol selections are necessary to enable deparaffinization and baking for the BenchMark Special Stains instrument.

Table 1. Recommended Staining Protocol for Green for Trichrome, using tissue cut at 3-5 µm thickness on a BenchMark Special Stains Automated Slide Stainer.

Staining Procedure: S Trichrome	
Protocol Step	**Method**
Deparaffinization	Select deparaffinization to automate paraffin removal.
Baking	Select temperature and incubation time to enable baking.
Bouin's	Select an incubation time up to 32 minutes. • 4 to 32 minute incubation time Bouin's may be selected as a secondary fixation step to correct pH of tissue and enable brighter dye staining.
Extended Bouin's (Optional)	Select an incubation time up to 32 minutes, if preferred. Time will be added to default Bouin's incubation time (32 minutes). • 4 to 32 minute incubation time Extended Bouin's may be selected if fixation of the tissue was especially problematic.
Optimize Stain Intensity **Hematoxylin Intensity (Hematoxylin A and B)**	Select Hematoxylin optimization: Option 1 enables dispense order of Hematoxylin A then B Option 2 enables dispense order of Hematoxylin B then A • 4 to 24 minute incubation time Ventana recommends selecting Option 1 for Hematoxylin dispense order and starting with a 4 minute incubation time. Option 2 may be used for brighter red staining.
Trichrome Red Intensity for Green (Trichrome Red)	Select an incubation temperature and time: • 37-60°C incubation temperature • 4 to 24 minute incubation time Ventana recommends starting at a 37°C incubation temperature and a 4 minute incubation time. If increased red clarity is preferred, Ventana recommends increasing the incubation temperature to 60°C and setting the incubation time to 8 minutes.
Trichrome Mordant for Green (Trichrome Mordant)	Select Mordant optimization (at least one is required): Mordant 1 (optional): • 37-60°C incubation • 4 to 24 minute incubation time Mordant 2 (optional): • 37-60°C incubation temperature • 4 to 32 minute incubation time Ventana recommends selecting both Mordant 1 and Mordant 2 and starting at a 37°C incubation temperature and a 4 minute incubation time for both Mordant steps.
Trichrome Green Intensity (Trichrome Green)	Select an incubation temperature and time: • No Heat for Green • 37-60°C incubation temperature • 4 to 32 minute incubation Ventana recommends starting at a 37°C incubation temperature and a 24 minute incubation time.

Table 2. Recommended Staining Protocol for Green for Trichrome, using tissue cut at 2 µm thickness on a BenchMark Special Stains Automated Slide Stainer.

Staining Procedure: S Trichrome	
Protocol Step	**Method**
Deparaffinization	Select deparaffinization to automate paraffin removal.
Baking	Select temperature and incubation time to enable baking.
Bouin's	Select an incubation time up to 32 minutes. • 4 to 32 minute incubation time Bouin's may be selected as a secondary fixation step to correct pH of tissue and enable brighter dye staining.
Extended Bouin's (Optional)	Select an incubation time up to 32 minutes, if preferred. Time will be added to default Bouin's incubation time (32 minutes). • 4 to 32 minute incubation time Extended Bouin's may be selected if fixation of the tissue was especially problematic.
Optimize Stain Intensity **Hematoxylin Intensity (Hematoxylin A and B)**	Select Hematoxylin optimization: Option 1 enables dispense order of Hematoxylin A then B Option 2 enables dispense order of Hematoxylin B then A • 4 to 24 minute incubation time Ventana recommends selecting Option 1 for Hematoxylin dispense order and starting with a 4 minute incubation time. Option 2 may be used for brighter red staining.
Trichrome Red Intensity for Green (Trichrome Red)	Select an incubation temperature and time: • 37-60°C incubation temperature • 4 to 24 minute incubation time Ventana recommends starting at a 60°C incubation temperature and a 24 minute incubation time.
Trichrome Mordant for Green (Trichrome Mordant)	Select Mordant optimization (at least one required): Mordant 1 (optional): • 37-60°C incubation • 4 to 24 minute incubation time Mordant 2 (optional): • 37-60°C incubation temperature • 4 to 32 minute incubation time Ventana recommends selecting only Mordant 1 and starting at a 50°C incubation temperature and a 4 minute incubation time.
Trichrome Green Intensity (Trichrome Green)	Select an incubation temperature and time: • No Heat for Green • 37-60°C incubation temperature • 4 to 32 minute incubation Ventana recommends starting at a 37°C incubation temperature and a 24 minute incubation time.

Recommended Post-Instrument Processing

1. Rinse slides in three changes of 95% alcohol to remove the coverslip solution.
2. Dehydrate, clear, and coverslip with permanent mounting media.

QUALITY CONTROL PROCEDURES

An example of a positive control material would be formalin-fixed, paraffin-embedded 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.

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.

LIMITATIONS

General Limitations

1. Histological staining is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Differences in tissue processing and technical procedures may produce significant variability of results necessitating regular performance of controls (see the Quality Control Procedures section). Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts or false negative results.
3. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. It is the responsibility of a qualified pathologist to be familiar with the special stain and methods used to produce the slide. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.

PERFORMANCE CHARACTERISTICS

BenchMark Special Stains Automated Slide Stainer

1. Instrument-to-Instrument: 100 slides of colon tissue were tested across 5 different BenchMark Special Stains instruments (4 slides per instrument/5 runs per day) on 5 non-consecutive days with Green for Trichrome. The slides were evaluated for staining with pass or fail criteria. The results demonstrated no significant difference in staining intensity among the slides.
2. Run-to-Run: 100 slides of colon tissue were tested across 5 BenchMark Special Stains instruments (4 slides per run/5 runs per day) on 5 non-consecutive days with Green for Trichrome. The slides were evaluated for staining with pass or fail criteria. The results demonstrated no significant difference in staining intensity among slides.
3. Lot-to-Lot: 3 different lots were tested on 3 different colon tissues. 20 slides per lot were tested across 1 BenchMark Special Stains instrument (20 slides per run/1 run per day) on 3 days with Green for Trichrome. The slides were evaluated for staining with pass or fail criteria. The results demonstrated no significant difference in staining intensity among slides.
4. Compatibility with Trichrome Staining Kit was tested with other selected special stains reagents. No significant adverse chemical interactions were observed.

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 does not stain appropriately, check to ensure the slide has the correct barcode label and it is applied correctly. If the label is correct, but no staining or unexpected staining occurs, contact your local support representative.
4. Ventana recommends incrementing the incubation temperature and time one parameter at a time to adjust staining preferences.

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 Edition. C.V. Mosby Company, St. Louis, 1980, p 190.
3. Lillie RD. Further experiments with the Masson Trichrome modification of Mallory's connective tissue stain. Stain Technol. 1940:15;82.
4. Clinical and Laboratory Standards Institute (CLSI). CLSI Web site. http://www.clsi.org/. Accessed November 3, 2011.

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