



## Trichrome III Green Staining Kit

Catalog number 860-023

### INDICATIONS AND USE

#### Intended Use

Ventana Medical Systems' special stains Trichrome III Green Staining Kit is intended for *in vitro* diagnostic use (IVD) as a qualitative histologic stain to study connective tissue, muscle and collagen fibers in formalin fixed, paraffin embedded tissue.

#### Summary and Explanation

Trichrome stains are used to differentiate collagen from muscle tissue.<sup>1</sup> The first trichrome system has been attributed to Mallory.<sup>2,3</sup> Further modifications were introduced by Masson and Gomori, and by Lillie.<sup>3,4</sup> 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 tumors that originated in fibroblasts.<sup>2</sup> Trichrome stains are usually nuclear and cytoplasmic stains, followed by a mordant, typically phosphotungstic or phosphomolybdic acid, followed by a collagen fiber stain.

Ventana's Trichrome III Green 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 III Red, containing Biebrich Scarlet and acid fuchsin. Nuclei are stained with iron hematoxylin. After application of Trichrome III Mordant, the collagen is stained with Trichrome III Green, which contains fast green FCF. The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests. Caution: U.S. Federal law restricts this device to sale by or on the order of a physician.

#### Principles and Procedures

This kit is optimized for use on the Ventana NexES<sup>®</sup> Special Stains automated slide stainer. 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 reagents for 75 tests.

1 - 27 ml vial of Trichrome III Bouin's Solution (1526800), containing 24.0% formaldehyde, 5% acetic acid and 71% picric acid-saturated solution

1 - 27 ml vial of Trichrome III Hematoxylin; containing 95% ethanol and 1% hematoxylin

1 - 27 ml vial of Trichrome III Hematoxylin B; containing 1.2% ferric chloride and 0.1% hydrochloric acid

1 - 27 ml vial of Trichrome III Enhancer; containing 0.5% lithium carbonate

1 - 27 ml vial of Trichrome III Red; containing 0.2% Biebrich Scarlet, 1% acetic acid, 0.05% Acid Fuchsin and 0.5% ethanol

1 - 27 ml vial of Trichrome III Red 2; containing 0.9% Biebrich Scarlet, 1% acetic acid and 0.1% Acid Fuchsin

1 - 27 ml vial of Trichrome III Mordant; containing 1% phosphomolybdic acid and 0.25% phosphotungstic acid

1 - 27 ml vial of Trichrome III Green; containing 0.5% Fast Green in 0.75% HCl

1 - 27 ml vial of Trichrome III Green 2; containing 1.0% Fast Green in 1.5% HCl

10 - vial inserts with sipping straws

#### Reconstitution, Mixing, Dilution, Titration

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

#### Materials and Reagents Needed But Not Provided

1. Neutral buffered formalin (refer to *Theory and Practice of Histotechnology* by Sheehan and Hrapchak)
2. Control tissue
3. Microtome
4. Microscope slides (positively charged)
5. Drying oven capable of maintaining a temperature of 70° C ± 5° C
6. Xylene (histological grade)
7. Alcohol or ethanol (histological grade)
8. Deionized or distilled water
9. Slide holders
10. Chemically clean laboratory glassware

11. Timer
12. Jars
13. Absorbent wipes
14. Synthetic mounting media
15. Cover glass
16. Ventana NexES Special Stains automated slide stainer
17. Ventana Special Stains 10X Wash Solution
18. Ventana Liquid Coverslip<sup>TM</sup> solution

#### Storage and Handling

The Trichrome III Green Staining Kit 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. The user must honor the printed expiration dates. 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 Ventana office should be contacted immediately.

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.

#### Specimen Collection and Preparation for Analysis

1. Ventana recommends specimen collection and storage be performed according to NCCLS document M29-T2.
2. Fix tissue blocks in 10% neutral buffered formalin.
3. Embed the fixed tissue blocks in paraffin.
4. Cut sections, usually 2 to 4 microns, and pick the sections up on glass slides.
5. Bake the slides for at least 30 minutes at approximately 70° C. Allow to cool.
6. Print appropriate barcode label(s).
7. Apply Trichrome III Green 1, 2, 3, 4, 5, 6, 7, 8 or 9 barcode labels to the frosted end of the slides prior to deparaffinization (see NexES Special Stains automated slide stainer Operator's Manual for correct application of labels).
8. The following protocol allows flexibility to accommodate varying tissue thickness, size and user preference. Trial runs using the protocols are suggested to tailor staining to the user's preference.

#### On line Bouins Protocols:

- TRI III GRN1 4µ liver and muscle sections
- TRI III GRN2 2µ and 4µ kidney sections with increased tubular staining
- TRI III GRN3 2µ and 4µ kidney sections with increased basement membrane staining
- TRI III GRN4 For increased muscle and collagen staining
- TRI III GRN5 2µ liver and gastric section

#### Off line Bouins Protocols:

- TRI III GRN6 2µ and 4µ liver sections
- TRI III GRN7 2µ kidney sections with increased basement membrane staining
- TRI III GRN8 2µ and 4µ kidney sections with increased tubular staining
- TRI III GRN9 For increased muscle and collagen staining

9. Deparaffinize and hydrate slides through xylenes and ethanols to deionized water following recommended procedures within the laboratory. Place tissue slides in Special Stains Wash Solution (1X) until loading the instrument. It is recommended that slides be loaded on the instrument within one hour. If not possible the slides may be left in deionized or distilled water for up to 24 hours as long as the tissue is placed in the refrigerator and not allowed to dry.

### WARNING AND PRECAUTIONS

1. The reagents in this kit have been optimally diluted for use on the NexES Special Stains automated slide stainer. These reagents may not be optimal for manual procedures or for use on other instruments. Further dilution may result in inadequate staining. The user must validate any change made to the reagent.
2. Differences in tissue processing and technical procedures may produce significant variability of results necessitating regular performance of controls (see Quality Control).
3. Normal precautions should be observed when handling laboratory reagents. Do not smoke, eat or drink in areas where specimens or reagents are being handled. Never pipette by mouth.
4. Disposal of liquid waste resulting from the use of this kit should be performed in accordance with all local, state and federal laws.

5. Patient specimens and all materials contacting them should be handled as if capable of transmitting infection. Use universal precautions when handling and disposing of specimens.
6. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
7. These reagents may cause irritation. Avoid contact with eyes and mucous membranes. If reagent contacts these areas, rinse with copious amounts of water.
8. Trichrome III Red contains acid fuchsin, a suspected carcinogen.
9. Trichrome III Enhancer contains lithium carbonate, a reproductive hazard.
10. Trichrome Mordant contains phosphotungstic acid, a corrosive substance.
11. Bouin's solution is toxic and contains formaldehyde, a compound that has been reported to be carcinogenic. Exposure may cause heritable genetic damage.
12. Iron Hematoxylin A is flammable, keep away from sources of ignition.

## INSTRUCTIONS FOR USE

### Step by Step Procedure

Refer to the NexES Special Stains automated slide stainer Operator's Manual for more detailed instructions.

1. Load reagents and slides onto the instrument.
2. Perform the staining run according to the instructions in the manual. Quantities of reagent to be applied are preprogrammed.
3. When the run is complete, remove the slides from the instrument, drain the slides and load into the slide holder.
4. Remove the residual coverslip solution by rinsing the slides in three changes of 95% alcohol.
5. Using routine laboratory methods, dehydrate through alcohol, clear in xylene, and apply mounting medium and cover glass.

### Quality Control Procedures

An example of a positive control material would be formalin fixed, paraffin embedded human tissue with collagen and smooth muscle present (colon, liver, esophagus 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. For this stain, a control block with two types of tissue may be desirable to ensure all relevant structures are stained. Since there are three stains included in the Trichrome III Green Staining Kit, virtually all elements in the control tissue will stain positively. The cellular components of other tissue elements may serve as the negative control.

The control tissue must be tested with each run of the Trichrome III Green Staining Kit. 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 Ventana office 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. 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. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and proper controls as well as other diagnostic tests. 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.
4. Ventana provides stains and reagents at optimal dilution for use when the provided instructions are followed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.

### Stability

Stained slides mounted using synthetic mounting media are stable when stored appropriately.

## SUMMARY OF EXPECTED RESULTS

1. Trichrome III Green Staining Kit stains collagen Green, muscle and erythrocytes red, and cell nuclei blue-black. The Trichrome III Green staining kit was tested across kidney, small intestine, liver, ureter, uterus, fallopian tube, appendix, skin, and heart tissue specimens. In 100% of the slides stained, the collagen stained green, muscle stained red and nuclei stained blue-black.
2. Sensitivity of the Trichrome III Green staining is dependent upon the type of fixative, degree of fixation and thickness of tissue section. Variations in these parameters can have a significant impact on the quality of staining and may result in inappropriate results.
3. Intra run reproducibility of staining with Trichrome III Green was determined by staining nine small intestine and nine kidney sections in one run. The staining intensity of collagen, muscle and nuclei were rated on a 1+ to 4+ for each component. The results were none of the individual components varied by more than one point across all 18 slides.
4. Inter run reproducibility was determined by taking the difference in staining intensity from each slide per case score between two runs (20 slides per run). The inter run intensity of staining of each tissue component did not vary by more than one point for all cases examined.

## TROUBLESHOOTING

1. Section thickness may affect quality and intensity of staining. If the staining is inappropriate, off line Bouin's pretreatment is recommended. Ventana recommends that the Bouin's should be preheated for 15 minutes at 60° C. Once the slides are added to the Bouin's reagent, incubate for another 1 hour at 60° C. Remove the slides from the Bouin's solution and wash in tap water for 10 minutes. Or, call your local Ventana office 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 Ventana office.

## REFERENCES

Lillie RD, Editor. H.J. Conn's Biological Stains, 9th ed. Lippincott Williams and Wilkins Company, Baltimore, 1977.

Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology, 2<sup>nd</sup> Edition. C.V. Mosby Company, St. Louis, 1980, p 190.

NCCLS documents can be obtained from NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA19087-1898, or through the web site [www.nccls.org](http://www.nccls.org).

Carson FL. Histotechnology: A Self Instructional Text, 2<sup>nd</sup> Edition. ASCP Press, Chicago, 1996.

Lillie RD. Further experiments with the Masson trichrome modification of Mallory's connective tissue stain. Stain Technol 15: 82, 1940.

## INTELLECTUAL PROPERTY

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Covered by the following patents: U.S. Pat. Nos. 6045759, 6192945B1, 6416713B1 and foreign counterparts. US and foreign patent applications pending.

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