Steiner II Staining Kit

REF 860-030

06521894001

IVD Σ 40

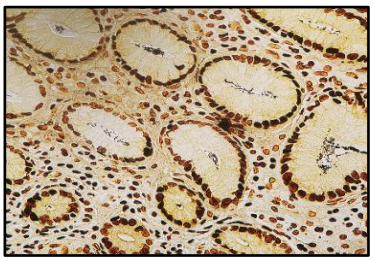

Figure 1. Steiner II Staining Kit staining H. pylori in gastric tissue.

INTENDED USE

The Steiner II Staining Kit is intended for laboratory use as a qualitative histologic stain to demonstrate argyrophilic microorganisms 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

The Steiner II Staining Kit is a modification of the Steiner silver stain. In 1944, Steiner and Steiner developed a modification to the Warthin and Starry silver impregnation technique to improve the efficiency and repeatability of pathogenic argyrophile detection.[1-3] Argyrophilic microorganisms such as Helicobacter pylori (H. pylori) and spirochetes can be identified using silver impregnation techniques.[2,4] Silver staining techniques are valuable because some microorganisms are difficult to visualize with hematoxylin and eosin (H&E).[2,5]

The Steiner II Staining Kit is used to aid the pathologist in the diagnosis of an infection with argyrophilic microorganisms.

PRINCIPLE OF THE PROCEDURE

The Steiner II Staining Kit uses silver nitrate to impregnate the microorganisms. The Steiner II Diffuser is applied to ensure that the enhancer will spread on the slide. The sections are exposed to the developer (Steiner II Reducer, Steiner II Enhancer and Steiner II Silver B), which allows the silver ions to be reduced by hydroquinone to black metallic silver and other tissue elements (background) to a yellow to amber color.[6]

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 40 tests:

One 19 mL vial of Steiner II Oxidizer contains 1.0 % zinc chloride, approximately 4% formaldehyde

One 15 mL vial of Steiner II Silver A contains 0.25% silver nitrate

One 15 mL vial of Steiner II Diffuser contains 50% reagent alcohol

One 15 mL vial of Steiner II Enhancer contains 5% gum mastic and absolute ethanol*

One 27 mL vial of Steiner II Clean A contains 95% reagent alcohol*

One 19 mL vial of Steiner II Reducer contains 0.8% hydroquinone

One 19 mL vial of Steiner II Silver B contains 0.20 % silver nitrate

One 27 mL vial of Steiner II Clean B contains 95% reagent alcohol**

Seven vial inserts with sipping straws

Cotton swabs

* Steiner II Clean A vial and Steiner II Enhancer vial must be next to each other in the reagent tray.

** Steiner II Clean B has no barcode label and is not intended to be inserted into the reagent tray.

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.

The reagents 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, uncharged
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. General purpose laboratory equipment

STORAGE AND STABILITY

The Steiner II Staining Kit 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 to the date indicated on the 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, formalin-fixed, paraffin embedded (FFPE) tissues are required for use with this product and BenchMark Special Stains instruments. The recommended tissue fixative is 10% neutral buffered formalin.[6]

Perform specimen collection and storage according to *Histotechnology: A Self Instructional Text*.[6] Cut sections to the appropriate thickness, approximately 4 µm, and place the sections on uncharged glass slides.

1. Dry the slides.[6]
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. 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.[7,8]
6. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
7. Avoid microbial contamination of reagents as it may cause incorrect results.
8. 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 navifyportal.roche.com.
9. Consult local and/or state authorities with regard to recommended method of disposal.
10. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.

11. 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
Danger 	H225	Highly flammable liquid and vapour.
	H302	Harmful if swallowed.
	H317	May cause an allergic skin reaction.
	H319	Causes serious eye irritation.
	H341	Suspected of causing genetic defects.
	H350	May cause cancer.
	H371	May cause damage to organs.
	H410	Very toxic to aquatic life with long lasting effects.
	P201	Obtain special instructions before use.
	P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
	P260	Do not breathe mist or vapours.
	P273	Avoid release to the environment.
	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.
	P308+ P311	IF exposed or concerned: Call a POISON CENTER/ doctor.
	P370+ P378	In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.
	P391	Collect spillage.

EUH208: Contains Hydroquinone. May produce an allergic reaction.

INSTRUCTIONS FOR USE

Prepare Reagent Vial

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

Note: Steiner II Clean B does not require a vial insert.

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 protocol 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 the Steiner II Staining Kit on a BenchMark Special Stains instrument.

Staining Procedure	S Steiner II
Protocol Step	**Method**
Deparaffinization	Select to automate paraffin removal
Baking (optional)	The default is not selected. • 75°C for 4 minutes is recommended.
No Pretreatment Dispense (optional)	The default is Pretreatment Dispense. Select No Pretreatment Dispense to skip the pretreatment steps for the assay, which consist of the Steiner II Oxidizer and the Steiner II Silver A components. Overall staining intensity and non-specific staining will be reduced when the pretreatment steps are skipped.
Developer Temperature	The recommended incubation time is 55°C. Select a temperature from 50-65°C: • 50°C, lighter staining of microorganisms * • 65°C, darker staining of microorganisms *
Optimize Stain Intensity	The default time is 12 minutes. Select to enable the adjustment of staining intensity. * Select an incubation time from 8-20 minutes: • 8 minutes, lighter staining of microorganisms • 20 minutes, darker staining of microorganisms

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

Recommended Post-Instrument Processing

1. Dehydrate slides in two changes of 95% ethanol to remove the leftover solution, followed by three changes of 100% ethanol.
2. Clear slides in three changes of 100% xylene.
3. Coverslip with permanent mounting media.
4. Compatible with the VENTANA HE 600 system coverslipping protocol. For further instructions, refer to VENTANA HE 600 system User Guide.

Special Cleaning Instructions

1. Open the aspirator cover.
2. Dip a cotton swab into the single vial labeled Steiner II Clean B.
3. Carefully swing out the arm of the aspirator mechanism.
4. Swab out the small metal cup below the aspirator tip.
5. Dip another swab into the Steiner II Clean B solution and carefully clean the tip of the aspirator.
6. Close the aspirator cover when the cleaning process is complete.

QUALITY CONTROL PROCEDURE

An example of a positive control material would be FFPE human tissue containing *H. pylori* or spirochetes. 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

The Steiner II Staining Kit is tested to demonstrate argyrophilic microorganisms.

- Organisms: Dark brown to black
- Nuclei: Brown to black
- Background: Yellow to amber

SPECIFIC LIMITATIONS

Failure to perform the special cleaning can lead to aspirator malfunction.

Only uncharged 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 for *H. pylori* and spirochetes infection was evaluated. All evaluated tissue slide cases (60/60) passed for acceptable staining as shown in Table 3.

Table 3. Sensitivity/Specificity of the Steiner II Staining Kit was determined by testing the following FFPE tissues.

Tissue	# Cases Passed / # Tested
Small intestine (normal)	23 / 23
H. pylori (stomach)	35 / 35
Spirochetes (kidney)	2 / 2

Precision

Precision of the Steiner II Staining Kit was determined across multiple runs, days, instruments and reagent lots using multiple cut slides from 10 stomach cases infected with *H. pylori* and 2 kidney cases infected with spirochetes. All acceptance criteria were fully met. Precision slide studies were performed according to Table 4.

Table 4. Precision slide studies for the Steiner II Staining Kit.

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

CLINICAL PERFORMANCE

The sensitivity and specificity characteristics relevant to the intended purpose of this device are reported in the analytical performance section. Additionally, published data relevant to the device were assessed by systematic review of the literature and also support the use of the device in accordance with its intended purpose.

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. 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 and proceed to post-instrument processing.
7. For corrective action, refer to the Instructions for Use section, the instrument User Guide or contact your local support representative.

REFERENCES

1. Garvey W. Silver Impregnation Techniques to Identify Spirochetes and Other Bacteria. J Histotechnol. 1996:19(3):203-209.
2. Bartlett J. Microorganisms In: Bancroft J, Gamble, M ed. Theory and Practice of Histological Techniques. 2008:309-331.
3. Woods GL, Walker DH. Detection of Infection or Infectious Agents by Use of Cytologic and Histologic Stains. Clinical Microbiology Reviews. 1996: 9(3):382-404.
4. Saiz E, Lubin J, Robinson MJ. The Modified Steiner Stain: A New Use for an Old Stain? Staining Cytomegalovirus-infected Cells in Gastrointestinal Biopsies. Histochem J. 1998;30(8):549-552.
5. Coleman T. Human Leptospirosis: Guidance for Diagnosis, Surveillance, and Control. 2003.
6. Carson FL, Cappellano C. Histotechnology; A Self-Instructional Text, 5th edition. American Society for Clinical Pathology Press; 2020, 2022.
7. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
8. Directive 2000/54/EC of the European Parliament and Council of 24 June 2020 on the protection of workers from risks related to exposure to biological agents at work.

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.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols 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
F	Updates to Material Provided and Specimen Preparation sections.

INTELLECTUAL PROPERTY

CONTACT INFORMATION

Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, Arizona 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)

www.roche.com

EC REP

Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany
+800 5505 6606