Giemsa Staining Kit

REF 860-006

05279224001

IVD Σ 75

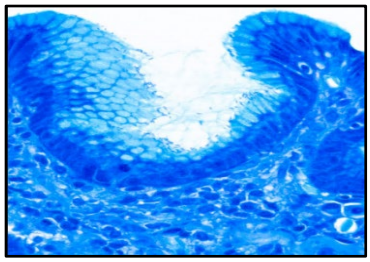

Figure 1. Giemsa Staining Kit staining *Helicobacter pylori*.

INTENDED USE

The Giemsa Staining Kit is intended for laboratory use as a qualitative histologic stain to demonstrate *Helicobacter pylori* in the stomach and differentiate hematopoietic cells by light microscopy in sections of formalin-fixed, paraffin-embedded (FFPE) tissue stained on 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 Giemsa Staining Kit utilizes a Giemsa stain that belongs to a group of neutral stains known as Romanowsky stains.[1,2] Giemsa is a versatile polychromatic stain which can be used in hematopoietic tissues to assess bone marrow/tissue cellularity and cellular differentials due to the differential binding of the dyes to the different cellular components.[3] In addition, the properties of the stain also enable the visualization of microorganisms, including *Helicobacter pylori*.[4] *H. pylori* are curved, highly motile, gram-negative bacteria, which generally colonize the stomach within the mucus layer overlying the epithelium.[5]

The Giemsa Staining Kit is used to aid the pathologist in the diagnosis of infection by *H. pylori* in the stomach.

PRINCIPLE OF THE PROCEDURE

The staining reaction is based on the differential affinity of cell types and organisms for the dyes in the stain. Because of the high degree of dissociation, active molecules (eosin and thiazine dyes) are absorbed by cellular structures very quickly.

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

MATERIAL PROVIDED

The reagent vial is supplied in a barcode labeled carrier to insert into the reagent tray of the instrument. Each kit contains sufficient reagent for 75 tests:

One 22 mL vial of Giemsa stain contains approximately 0.4% modified Giemsa stain in 70% methanol.

One 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.

The reagent in this kit has 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. General purpose laboratory equipment.

STORAGE AND STABILITY

The Giemsa Staining Kit should be stored at 15-30°C.

When properly stored, unopened and opened reagent is 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 the reagent; 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.[6]

Perform specimen collection and storage according to CLSI document M29-T2.[7] Cut sections to the appropriate thickness, approximately 3 µm, and place the sections on positively charged 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 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.[8,9]
7. Avoid contact with eyes and mucous membranes. If reagent comes in contact with sensitive areas, wash 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
Danger 	H225	Highly flammable liquid and vapour.
	H301+ H311+ H331	Toxic if swallowed, in contact with skin or if inhaled.
	H370	Causes damage to organs.
	P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
	P260	Do not breathe mist or vapours.
	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.
	P301+ P310+ P330	IF SWALLOWED: Immediately call a POISON CENTER/ doctor. Rinse mouth.
	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.
	P403+ P233	Store in a well-ventilated place. Keep container tightly closed.

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 protocol (see 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 Giemsa Staining Kit on a BenchMark Special Stains instrument.

Staining Procedure	S Giemsa
Protocol Step	**Method**
Deparaffinization	Select to automate paraffin removal.
Baking (optional)	The default is not selected. 75°C for 4 minutes is recommended.

Recommended Post-Instrument Processing

Stomach

1. Dip slides in two changes of 95% alcohol for 15 to 45 seconds each, with gentle agitation.
2. Dip slides in three changes of absolute alcohol for 5 seconds each, with gentle agitation.
3. Dip slides in three changes of xylene for 5 seconds each, with gentle agitation.
4. 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.

Other Tissue Types

1. Dip slides in 0.1% acetic acid for 3 to 5 seconds.
2. Dip slides in three changes of absolute alcohol for 5 seconds each, with gentle agitation.
3. Dip slides in three changes of xylene for 5 seconds each, with gentle agitation.
4. Coverslip with permanent mounting media.

QUALITY CONTROL PROCEDURE

An example of a positive control material would be FFPE human tissue such as bone marrow, lymph node, spleen, or stomach with *H. pylori*.[6] 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. 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

Giemsa Staining Kit is tested to demonstrate *H. pylori* in stomach tissue and differentiate hematopoietic cells in other tissues.

Stomach

- *H. pylori*: blue

Other Tissues

- Erythrocytes: pink to red
- Eosinophilic granules: red
- Monocytes, lymphocytes, mast cells: blue to purple

SPECIFIC LIMITATIONS

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

Only *H. pylori* infected stomach was tested with the VENTANA HE 600 system coverslipping protocol.

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 for normal and diseased tissue cases cut at approximately 3 µm. All evaluated tissue cases (63/63) passed for acceptable staining as shown in Table 3 and Table 4.

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

Tissue	# passed / # tested
Bone marrow*	14 / 14
Spleen	9 / 9
Skin	4 / 4
Lymph node	8 / 8
Stomach	6 / 6

*Unspecified decalcification

Table 4. Sensitivity/Specificity of Giemsa Staining Kit was determined by testing the following FFPE diseased tissues.

Tissue	# passed / # tested
Leukemia (bone marrow*)	6 / 6
H. pylori (stomach)	16 / 16

*Unspecified decalcification

Precision

Precision of Giemsa Staining Kit was determined across multiple runs, days, instruments, and reagent lots using multiple cut slides from 2 normal spleen tissue cases and 4 stomach cases with *H. pylori*. Test cases were cut at approximately 3 µm. All acceptance criteria were fully met. Precision studies were performed for the Giemsa Kit according to Table 5.

Table 5. Precision slide studies for Giemsa Staining Kit.

Parameters Tested	# of conditions	# 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.

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 support the use of the device in accordance with its intended purpose.

TROUBLESHOOTING

1. Section thickness may affect quality and intensity of staining. If the 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 is labeled properly. 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. 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.
8. 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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7. Clinical and Laboratory Standards Institute (CLSI). CLSI Web site. http://www.clsi.org/. Accessed November 3, 2011.
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9. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 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.

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
G	Updates to Specimen Preparation and Hazard information

INTELLECTUAL PROPERTY

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