VENTANA®

Roche

Gram Staining Kit

REF 860-039

06890105001

IVD Σ 75

INTENDED USE

Gram Staining Kit is intended for use as a qualitative histologic stain to demonstrate gram-negative and gram-positive bacteria 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

Gram Staining Kit is a modification of the original Gram stain.[1-3] Gram-negative bacteria stain pink to red and gram-positive bacteria stain blue to dark purple.

PRINCIPLE OF THE PROCEDURE

This kit is optimized for use on the VENTANA BenchMark Special Stains instrument. The reagents are applied to tissue on microscope slides and mixed over the entire specimen. The staining reaction for Gram Staining Kit is based on the formation of a dye lake (crystal violet-iodine complex) that penetrates the bacteria cell walls. The microscope slide is differentiated with SSR Solution, which removes the dye from some tissue elements but not from the gram-positive bacteria. Following the differentiation step, the tissue is stained with basic fuchsin and Gallego solution. The tissue can be counterstained with tartrazine or fast green, or no counterstain can be selected.

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 Gram Crystal Violet contains 1% crystal violet, 0.7% ammonium oxalate monohydrate, and 17.5% reagent alcohol.

One 27 mL vial of Gram Iodine contains 0-15% Povidone iodine and <2% stabilized gram iodine.

One 27 mL vial of Gram Basic Fuchsin contains 0.15% basic fuchsin.

One 27 mL vial of Gram Gallego contains approximately 1.2% formaldehyde and 1.5% acetic acid.

One 27 mL vial of Gram Tartrazine contains 0.1% tartrazine and 0.25% acetic acid.

One 27 mL vial of Gram Fast Green contains 0.002% fast green and 0.025% acetic acid.

6 vial inserts with sipping straws.

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

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 instrument
5. Bulk reagents for the BenchMark Special Stains instrument:
   - BenchMark Special Stains Deparaffinization Solution (10X) (Cat. No. 860-036 / 06523102001)
   - BenchMark Special Stains Liquid Coverslip (Cat. No. 860-034 / 06523072001)
   - BenchMark Special Stains Wash II (Cat. No. 860-041 / 08309817001)
   - SSR Solution (Cat. No. 860-038 / 06890059001)
6. Xylene or other clearing reagent (Histological grade)
7. Reagent alcohol or ethanol (Histological grade)
8. Deionized or distilled water
9. Synthetic mounting media
10. Coverslip

STORAGE AND STABILITY

The Gram Staining Kit should be stored at 15-30°C. See vial label for proper storage conditions.

When properly stored, unopened 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

Ventana recommends specimen collection and storage be performed according to Histotechnology; A Self-Instructional Text.[1] The recommended tissue fixative is 10% neutral buffered formalin.[3]

Cut sections, usually 3 to 5 µm, and pick up the sections on glass slides.

1. Cut sections of 3 µm are recommended to reduce background and improve clarity of the gram-positive and gram-negative bacteria.
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 User Guide for correct application of labels).
5. 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. Do not use beyond the specified number of tests.
4. 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.
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.[4,5]
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. Contamination could produce erroneous results.
8. Gram Crystal Violet contains reagent alcohol which is flammable and contains ammonium oxalate monohydrate which may cause cancer. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
9. Gram Basic Fuchsin includes basic fuchsin which may cause cancer.
10. Gram Gallego includes formaldehyde which has limited evidence of a carcinogenic effect. May cause sensitization by skin contact.
11. 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.
12. Consult local and/or state authorities with regard to recommended method of disposal.
13. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.

14. 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 	H226	Flammable liquid and vapour.
	H317	May cause an allergic skin reaction.
	H319	Causes serious eye irritation.
	H341	Suspected of causing genetic defects.
	H350	May cause cancer.
	H411	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.
	P273	Avoid release to the environment.
	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.
	P308 + P313	IF exposed or concerned: Get medical advice/ attention.
	P370 + P378	In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.
	P391	Collect spillage.

EUH208: Contains trisodium 5-hydroxy-1-(4-sulphophenyl)-4-(4-sulphophenylazo)pyrazole-3-carboxylate. May produce an allergic reaction.

This product contains CAS #s

- 548-62-09: [4-[4,4'-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride
- 50-00-0: formaldehyde

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 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 varying tissue thickness, size and user preference. Trial runs using the protocols are suggested to adjust staining to the user's preference. This product has been optimized for use with BenchMark Special Stains instruments but the user must validate results obtained with this product.

In the protocol in Table 2, the protocol selections are necessary to enable deparaffinization and baking for the BenchMark Special Stains instrument.

Table 2. Recommended staining protocol for Gram Staining Kit on a BenchMark Special Stains instrument.

Protocol Step	Method
Deparaffinization	Select deparaffinization to automate paraffin removal.
Baking	Select temperature and incubation time to enable baking.
Counterstain	Choose Counterstain: • Tartrazine Counterstain • Fast Green Counterstain • No Counterstain After counterstain is selected, Gram + and Gram – Intensities can be adjusted.
Optimize Stain Intensity Gram + (Optional)	Choose incubation time from 4 to 16 minutes: • Lighter staining 16 minutes incubation time. • Darker staining 4 minute incubation time. NOTE: If Gram-positive bacteria are staining too dark, increase the incubation time to increase the amount of differentiation and reduce the staining intensity. If Gram-positive bacteria are staining too light, reduce the incubation time to decrease the amount of differentiation and increase the staining intensity.
Optimize Stain Intensity Gram – (Optional)	Choose incubation time from 4 to 12 minutes: • Lighter staining 4 minute incubation time. • Darker staining 12 minutes incubation time. NOTE: If Gram-negative bacteria are staining too dark, decrease the incubation time to reduce the staining intensity. If Gram-negative bacteria are staining too light, increase the incubation time to increase the staining intensity.

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 3 changes of 100% xylene.
3. Coverslip with permanent mounting media.

QUALITY CONTROL PROCEDURE

Control tissue(s) must be tested with each run.

Control tissue is formalin-fixed, paraffin-embedded (FFPE) human tissue with both gram-negative bacteria and gram-positive bacteria. Control tissue may be one or more tissue samples on one or more slides.

Control tissue(s) 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.

Gram Staining Kit assay stains gram-positive bacteria blue to dark purple and gram-negative bacteria pink to red, and the background tissue is counterstained either yellow or green.

If the control tissue(s) fails to demonstrate both gram positive and gram negative bacteria staining, results should be considered invalid.

The control tissue must be tested with each run.

Known 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.

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.

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 information, 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 tissue controls.
4. Antibiotics can affect the cell wall of gram-positive bacteria and therefore affect the staining quality of the Gram Staining Kit.
5. If you are evaluating for Nocardia, you may prefer an alternative method for visualizing this bacteria. Nocardia, a variable gram positive bacteria, may stain pink to red with some blue to purple segments.
6. Interpretation of equivocal staining results should be performed in correlation with other clinical tests.

PERFORMANCE CHARACTERISTICS

BenchMark Special Stains Instrument

1. Instrument-to-Instrument: 120 slides of gram positive and gram negative infected tissue were tested across 5 different BenchMark Special Stains instruments with the Gram Staining Kit. 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: 96 slides of gram positive and gram negative infected tissue were tested across 4 BenchMark Special Stains instruments (4 slides per run/1 run per day) on 6 days with the Gram Staining Kit. 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 gram positive and gram negative infected tissue. 20 slides per lot were tested with the Gram Staining Kit. The slides were evaluated for staining with pass or fail criteria. The results demonstrated no significant difference in staining intensity among slides.
4. The performance of the Gram Staining Kit was tested with 15 different tissue cases.
5. Compatibility with Gram Staining Kit was tested with other special stains reagents. No significant adverse chemical interactions were observed.

TROUBLESHOOTING

1. Section thickness may affect quality and intensity of staining. Refer to Specimen Collection and Preparation for Analysis for recommended section thickness. 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 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.
6. 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.
7. If tissue sections wash off the slide, confirm the slides are positively charged.
8. Extended stay of the slides on 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.
9. For corrective action, refer to the Instructions for Use section, the instrument User Guide or contact your local support representative.

REFERENCES

1. Carson FL, Cappellano C. Histotechnology; A Self-Instructional Text, 5th edition. American Society for Clinical Pathology Press; 2020, 2022.
2. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology. 2nd ed. St. Louis, MO: C.V. Mosby Company; 1980.
3. Bancroft JD, Gamble, M. Theory and Practice of Histological Techniques. 2nd ed. Edinburgh: Churchill-Livingston; 1982.
4. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
5. 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.

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 more information).

GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
G	Updates to Warnings and Precautions section.

INTELLECTUAL PROPERTY

For USA: Rx only

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