

Light Green for PAS

REF

860-010

05279267001

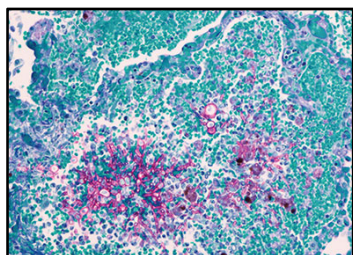
IVD
 75


Figure 1. Light Green for PAS staining of fungal organisms in lung tissue.

diagnostic (IVD) use.

SUMMARY AND EXPLANATION

Light Green for PAS is a single bottle counterstain used in conjunction with the PAS Staining Kit. The PAS Staining Kit is a modification of a technique originally described by McManus in 1946 to visualize mucins, glycogen, basement membrane and fungal organisms through the combination of oxidation of polysaccharides by periodic acid and staining with the Schiff's reagent.¹

PRINCIPLE OF THE PROCEDURE

The PAS Staining Kit uses Periodic Acid reagent to oxidize glycols to aldehydes. The Schiff's Reagent forms a colorless dialdehyde compound that is transformed to the magenta staining of glycol containing cellular components.² Light Green for PAS reagent is used instead of or alongside the Hematoxylin supplied with the PAS Staining Kit. Light Green for PAS stains the background light green while the PAS Staining Kit stains the fungal tissue elements magenta.

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

MATERIAL PROVIDED

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

One 15 mL vial of Light Green for PAS contains approximately 1% light green SF yellowish in a 1% acetic acid solution.

One vial insert with sipping straw.

Reconstitution, Mixing, Dilution, Titration

No reconstitution, mixing, dilution, or titration of kit reagent is required. Further dilution of the reagent may result in unsatisfactory staining quality.

The reagent in this kit has been optimally diluted for use on the BenchMark Special Stains instrument.

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. PAS Staining Kit (Cat. No. 860-014 / 05279291001)

8. General purpose laboratory equipment

STORAGE AND STABILITY

Light Green for PAS should be stored at 15-30°C.

When properly stored, opened and unopened reagents are stable to the date indicated on the label. Do not use reagent beyond the expiration date.

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 assay and BenchMark Special Stains. The recommended tissue fixative is 10% neutral buffered formalin.²

Perform specimen collection and storage according to CLSI document M29-T2.³ Cut sections to the appropriate thickness, approximately 4 µm, and place the sections on positively charged glass slides.

1. Dry the slides.²
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. 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.^{4,5}
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. Consult local and/or state authorities with regard to recommended method of disposal.
10. 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.
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.

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 1, 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 BenchMark Special Stains instruments but the user must validate results obtained with this product.

Table 1. Recommended staining protocol for Light Green for PAS on a BenchMark Special Stains instrument.

Staining Procedure	S PAS
Protocol Step	Method
Deparaffinization	Select to automate paraffin removal.
Baking (optional)	The default is not selected. 75°C for 4 minutes is recommended.
Light Green	The default is not selected. Select to enable Light Green and options for Light Green.
Diastase for PAS Light Green* (optional)	Not selected. This option must be validated by the user.
Optimize Schiff's for PAS (PAS Schiff's)	The default is 45°C for 20 minutes. Select to enable adjustment of staining intensity.** Select a temperature from 37-60°C: 37°C, lighter Schiff's staining intensity 60°C, darker Schiff's staining intensity Select an incubation time from 12 to 20 minutes: 12 minutes, lighter Schiff's staining intensity 20 minutes, darker Schiff's staining intensity
Hematoxylin for Light Green (optional)	The default is not selected. Select to enable the application of Hematoxylin nuclear stain (recommended).

* Additional procedure options are available for products that can be used in conjunction with the PAS staining Kit and Light Green for PAS.

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

Recommended Post-Instrument Processing

1. Rinse slides in two changes of 95% ethanol to remove the leftover solution, followed by three changes in 100% ethanol.
2. Dehydrate slides in three changes of 100% xylene.
3. 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.

QUALITY CONTROL PROCEDURE

An example of a positive control material would be FFPE human tissue known to be positive for fungal organisms. 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

Light Green for PAS, used in conjunction with the PAS Staining Kit, is tested to provide a contrasting background to aid in the demonstration of fungal organisms.

- Fungal organisms: magenta
- Nuclei: blue-green
- Background: light green

SPECIFIC LIMITATIONS

Only positively charged 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 normal and diseased tissue cases was evaluated. All evaluated tissue cases (69/69) passed for acceptable staining as shown in Table 2 and Table 3.

Table 2. Sensitivity/Specificity of Light Green for PAS was determined by testing the following FFPE normal tissues.

Tissue	# passed / # tested
Lung	1 / 1
Skin	4 / 4

Table 3. Sensitivity/Specificity of Light Green for PAS was determined by testing the following FFPE diseased tissues.

Tissue	# passed / # tested
Candida (various tissue types)	8 / 8
Aspergillus (various tissue types)	43 / 43
Blastomyces (various tissue types)	3 / 3
Mucormycosis (various tissue types)	5 / 5
Coccidioidomycosis (Lung)	2 / 2
Histoplasmosis (various tissue types)	3 / 3

Precision

Precision of Light Green for PAS was determined across multiple runs, days, instruments, and reagent lots using multiple cut slides from 3 Aspergillus infected lung tissue cases and 3 Candida infected esophageal tissue cases. All acceptance criteria were fully met. Precision studies were performed for Light Green for PAS according to Table 4.

Table 4. Precision slide studies for Light Green for PAS.

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.

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. 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

1. Layton C, Bancroft JD. Bancroft's Theory and Practice of Histological Techniques. In: Elsevier; 2019. Accessed 02/15/2021.
2. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
3. Clinical and Laboratory Standards Institute (CLSI). CLSI Web site. <http://www.clsi.org/>. Accessed November 3, 2011.
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 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):



Global Trade Item Number



Unique Device Identification



Indicates the entity importing the medical device into the European Union

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CONTACT INFORMATION

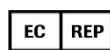


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