VENTANA®

Roche

PAS Staining Kit

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IVD Σ 75

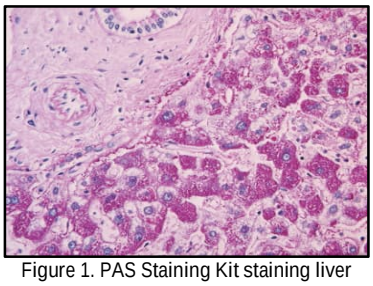

Figure 1. PAS Staining Kit staining liver tissue.

INTENDED USE

The PAS Staining Kit is intended for laboratory use as a qualitative histologic stain to demonstrate the presence of glycogen, basement membrane and fungal organisms by light microscopy in sections of formalin-fixed, paraffin-embedded tissue (FFPE) 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 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 reagent.[1]

PAS is a versatile carbohydrate stain that can be used to detect structures containing a high proportion of carbohydrate macromolecules including polysaccharides.[1] The main polysaccharide identified via histologic staining in human tissue sections is glycogen.[1]

PAS staining enables microscopic visualization and assessment of glycogen levels in tissue.[2] Disruption in metabolic pathways that convert glucose to glycogen and vice versa can lead to glycogen accumulation.[2] The clinical features of excess glycogen can vary depending on the organ/tissue where the buildup occurs.[2]

The PAS stain is also used in the examination of basement membranes and is typically used to inspect the glomerular basement membrane in renal biopsies suspected of glomerular disease.[3,4] Glomerular disease occurs when there is a disruption to the glomerular filtration barrier that may allow red blood cells or plasma proteins to pass through the barrier.[5] Pathological features can vary but can include corrugation, collapse, thickening and thinning of the basement membrane.[4]

The PAS stain can also be used to detect glycogen present in fungal cell walls as an aid in diagnosis of fungal infection.6 The glycogen content of the fungal cell wall is similar across many fungi; therefore PAS staining is a reliable method to confirm or eliminate the possibility of fungal infection.[1,6]

The PAS Staining Kit is used to aid the pathologist in the assessment of levels of glycogen and to aid in the diagnosis of glomerular disease in kidney tissue and infection by fungal organisms.

PRINCIPLE OF THE PROCEDURE

The PAS Staining Kit uses Periodic Acid reagent to oxidize glycols to aldehydes.[1] The Schiff's reagent reacts with aldehydes to form a colorless dialdehyde compound that is transformed to the magenta staining of glycol containing cellular components. A modification of Mayer's hematoxylin is applied to provide blue to purple nuclear staining.

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 75 tests:

One 22 mL vial of Periodic Acid contains approximately 1% periodic acid.

Three 22 mL vials of Schiff's Reagent contains approximately 4% sodium bisulfite, approximately 2% dilute hydrochloric acid, and approximately 1% pararosaniline chloride.

One 22 mL vial of Neutralizer contains approximately 1% sodium bisulfite.

One 22 mL vial of Hematoxylin Counterstain contains modified Mayer's hematoxylin.

Six 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

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 PAS 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 reagents are stable to the date indicated on the label. Do not use reagent beyond the expiration date indicated on the kit.

Note: Schiff's Reagent is stable for one month after opening. Label the Schiff vial with the corresponding date when first opened. The kit contains two extra bottles of Schiff's Reagent to allow full use of the kit. Use only one vial of Schiff's Reagent at a time.

Schiff's Reagent is known to contain needle-like precipitate. At expected levels, this precipitate should not affect assay performance. 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.[7]

Perform specimen collection and storage according to Histotechnology: A Self Instructional Text.[7] Cut sections to the appropriate thickness, approximately 4 µm, and place the sections on positively charged glass slides.

1. Dry the slides.[7]
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. 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.[8,9]
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 	H290	May be corrosive to metals.
	H314	Causes severe skin burns and eye damage.
	H350	May cause cancer.
	H373	May cause damage to organs through prolonged or repeated exposure.
	P201	Obtain special instructions before use.
	P260	Do not breathe mist or vapours.
	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.
	P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
	P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
	P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.
	P308 + P313	IF exposed or concerned: Get medical advice/ attention.

EUH208: Contains Sodium iodate. 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.

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

Table 2. Recommended staining protocol for PAS Staining Kit 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.
Optimize PAS	Select to enable adjustments of PAS Schiff's and Hematoxylin.
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 staining intensity 60°C, darker Schiff staining intensity Select an incubation time from 12-20 minutes: • 12 minutes, lighter Schiff's staining intensity 20 minutes, darker Schiff's staining intensity.
PAS Hematoxylin	The default is 8 minutes. Select to enable adjustment of incubation time:** 4 minutes, lighter nuclear staining 12 minutes, darker nuclear staining

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

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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.
4. 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 glycogen rich, such as skeletal muscle or liver. 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 PAS Staining Kit is tested to demonstrate glycogen, fungus and basement membranes.

- Glycogen: magenta
- Mucins:[7] magenta
- Basement membrane: magenta
- Fungi: magenta
- Nuclei: blue to purple
- Background: pink

SPECIFIC LIMITATIONS

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

Schiff's Reagent is known to contain needle-like precipitate. At expected levels, this precipitate should not affect assay performance.

Pink to red, cube-shaped precipitate has been observed on slides stained with PAS Staining Kit. At expected levels, this precipitate should not interfere with the interpretation of the 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 (61/61) passed for acceptable staining as shown in Table 3 and Table 4.

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

Tissue	# cases passed / # tested
Kidney	5 / 5
Liver	5 / 5
Small intestine	5 / 5
Skeletal muscle	10 / 10

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

Tissue	# cases passed / # tested
Candida (various tissue types)	8 / 8
Aspergillus (various tissue types)	10 / 10
Focal segmental glomerulosclerosis (Kidney)	4 / 4
Glomerular disease (Kidney)	5 / 5
Membranous glomerulonephritis (Kidney)	1 / 1
Membranoproliferative glomerulonephritis (Kidney)	2 / 2
Lupus nephropathy (Kidney)	4 / 4
Diabetic glomerulosclerosis (Kidney)	2 / 2

Precision

Precision of the PAS Staining Kit was determined across multiple runs, days, instruments, and reagent lots using multiple cut slides from 3 normal liver and 3 normal kidney tissue cases. All acceptance criteria were fully met. Precision studies were performed for the PAS Staining Kit according to Table 5.

Table 5. Precision slide studies for PAS Staining Kit.

Parameters Tested	# of conditions	# slides passed / # tested
Run to Run	3 runs, same day	54 / 54
Day to Day	5 days	88 / 90
Instrument to Instrument	3 instruments	52 / 54
Intra Run	same day, same instrument	53 / 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. If tissue sections wash off the slide, confirm the slides are positively charged.
7. 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.
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. Kanungo S, Wells K, Tribett T, et al. Glycogen Metabolism and Glycogen Storage Disorders. Ann Transl Med. 2018;6(24):474.
3. McManus JFA. The Periodic Acid Routine Applied to the Kidney. Am J Pathol. 1948:643-653.
4. Cathro HP, Shen SS, Truong LD. Diagnostic Histochemistry in Medical Diseases of the Kidney. Semin Diagn Pathol. 2018;35(6):360-369.
5. Hebert LA, Parikh S, Prosek J, et al. Differential Diagnosis of Glomerular Disease: A Systematic and Inclusive Approach. Am J Nephrol. 2013;38(3):253-266.
6. Guarner J, Brandt ME. Histopathologic Diagnosis of Fungal Infections in the 21st Century. Clin Microbiol Rev. 2011;24(2):247-280.
7. Carson FL, Cappellano C. Histotechnology; A Self-Instructional Text, 5th edition. American Society for Clinical Pathology Press; 2020, 2022.
8. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
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 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
K	Updates to Warnings and Precautions section and updated to current template.

INTELLECTUAL PROPERTY

For USA: Rx only

CONTACT INFORMATION

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