

Alcian Blue for PAS



860-003

05279194001



INTENDED USE

Alcian Blue for PAS Staining Kit, in conjunction with the PAS Staining Kit (860-014 / 05279291001), is intended for use as a qualitative histologic stain to differentiate acid mucin from neutral polysaccharides 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

Alcian Blue for PAS Staining Kit uses periodic acid to oxidize glycols to aldehydes. Schiff's reagent forms a colorless dialdehyde compound that is transformed to the colored final staining of glycol containing cellular components.¹ The Alcian Blue for PAS Staining Kit can also be used with the Diastase Kit in conjunction with the PAS Staining Kit. Alcian Blue for PAS Staining Kit has a pH of 2.5. At this pH, it stains both sulfated and carboxylated acid mucopolysaccharides, such as in goblet cells. It also stains sulfated and carboxylated sialomucins, such as are seen in salivary glands, goblet cells and gastric lining cells. The hyaluronic acid in umbilical cord and dermal tissue also stains.²

PRINCIPLE OF THE PROCEDURE

This kit is optimized for use on VENTANA BenchMark Special Stains automated slide stainers and VENTANA NexES Special Stains automated slide stainers. The reagents are applied to tissue on microscope slides and mixed over the entire specimen. The staining reaction is based on the oxidation of glycol to aldehyde followed by selective staining of the aldehyde groups by Schiff's reagent. The acidic Alcian Blue reagent differentially stains acid mucins and neutral mucopolysaccharides.

MATERIALS AND METHODS

Reagent Provided

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

One 22 mL vial of Alcian Blue contains 1.2% alcian blue in a 3% acetic acid solution.

1 vial insert with sipping straw.

Reconstitution, Mixing, Dilution, Titration

No reconstitution, mixing, dilution, or titration of kit reagent is required. Further dilution of any of the reagents may result in unsatisfactory staining quality. The user must validate any such change.

The reagent in this kit has been optimally diluted for use on BenchMark Special Stains automated slide stainers and NexES Special Stains automated slide stainers. This reagent may not be optimal for manual procedures or for use on other instruments.

Materials and Reagents Needed But Not Provided

- Control tissue
- Microscope slides (positively charged)
- Drying oven capable of maintaining a temperature of 70°C ± 5°C
- BenchMark Special Stains automated slide stainer or NexES Special Stains automated slide stainer
- Bulk reagents for the BenchMark Special Stains automated slide stainer:
 - BenchMark Special Stains Deparaffinization Solution (10X)
 - BenchMark Special Stains Liquid Coverslip
 - BenchMark Special Stains Wash Solution (10X)
- Bulk reagents for the NexES Special Stains automated slide stainer:
 - Liquid Coverslip (Low Temperature)
 - Special Stains Wash (10X)
- PAS Staining Kit (860-014 / 05279291001)
- Diastase Staining Kit (860-004 / 05279208001)

- Xylene or other clearing reagent (Histological grade)
- Reagent alcohol or ethanol (Histological grade)
- Deionized or distilled water
- Synthetic mounting media
- Coverslip

Storage and Handling

The Alcian Blue for PAS Staining Kit should be stored at 15-30°C. See vial label for proper storage conditions. Refrigerated kit components should be brought to room temperature prior to use.

When properly stored, reagents are stable until the expiration date that is printed on the vial label. There are no obvious signs to indicate instability of these reagents; therefore, controls should be run simultaneously with unknown specimens. If positive control material shows a decrease in staining it could indicate reagent instability and your local support representative should be contacted immediately.

Specimen Collection and Preparation for Analysis

Ventana recommends specimen collection and storage be performed according to CLSI document M29-T2.³ The recommended tissue fixative is 10% neutral buffered formalin.⁴

- Cut sections, usually 3 to 5 µm, and pick up the sections on glass slides.
- 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.
- Print appropriate barcode label(s).
- Apply barcode labels to the frosted end of the slides prior to deparaffinization (see the instrument Operator's Manual for correct application of labels).
- Refer to the Instructions for Use section for the recommended protocol for the BenchMark Special Stains automated slide stainer and NexES Special Stains automated slide stainer.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic (IVD) use.
- Consult local and/or state authorities with regard to recommended method of disposal.
- Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. Use universal precautions when handling and disposing of specimens.
- Avoid microbial contamination of reagents. Contamination could produce erroneous results.
- This reagent may cause irritation. Avoid contact with eyes and mucous membranes. If reagent contacts these areas, rinse with copious amounts of water. Do not ingest or inhale any reagents.
- For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Risk Phrase Guide located at www.ventana.com.

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.
- Place the soft cap into the slot on the reagent holder when the reagent is in use.
- Use the soft cap to cover the reagent vial when reagent is not in use.

Staining Procedure

- Load reagents and slides onto the instrument.
- Perform the staining run according to the recommended protocol (see Table 1 or Table 2) and the instructions in the manual.
- When the run is complete, remove the slides from the instrument.

Recommended Protocol

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument Operator's Manual.

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.

In the protocol in Table 1, the protocol selections are necessary to enable deparaffinization and baking for the BenchMark Special Stains instrument.

Table 1. Recommended Staining Protocol for Alcian Blue for PAS Staining Kit on a BenchMark Special Stains Automated Slide Stainer using the PAS protocol.

Protocol Step	Method
Deparaffinization	Select deparaffinization to automate paraffin removal.
Baking	Select temperature and incubation time to enable baking.
Alcian Blue	Select to enable Alcian Blue options.
Diastase for PAS AB (PAS Diastase) OPTIONAL	Select Optimize Diastase for PAS AB and then select an incubation time from 4 to 16 minutes. Staining intensity will vary according to incubation time.
Optimize Stain Intensity (PAS Alcian Blue)	Select an incubation time from 8 to 16 minutes: <ul style="list-style-type: none"> 8 minutes, lighter blue mucosubstances 16 minutes, darker blue mucosubstances
Hematoxylin for Alcian Blue (Optional - PAS Hematoxylin)	Select an incubation time from 4 to 12 minutes: <ul style="list-style-type: none"> 4 minutes, lighter counterstain 12 minutes, darker counterstain
No Counterstain	If no Hematoxylin counterstain is required, do not select Hematoxylin for Alcian Blue.

Table 2. Recommended Staining Protocol for Alcian Blue for PAS Staining Kit (PAS Flex) on a NexES Special Stains Automated Slide Stainer.

Options	
Temperature	37°C or 60°C
Stain	Alcian Blue Diastase Hematoxylin Light Green
Schiff's Time for 60°C	4 to 8 minutes
Diastase Time	4 to 32 minutes
Hematoxylin Intensity	Light or Dark
Pre-Programmed Protocol	
37°C protocols	Have protocol numbers starting with 3XX
60°C protocols	Have protocol numbers starting with 6XX and 7XX

Recommended Post-Instrument Processing

- Rinse slides in two changes of 95% alcohol to remove the coverslip solution.
- Dehydrate, clear, and coverslip with permanent mounting media.

QUALITY CONTROL PROCEDURES

An example of a positive control material would be formalin-fixed, paraffin-embedded human tissue with acid mucin and neutral polysaccharides present (colon, small intestine, or salivary glands).⁴ 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.

The control tissue must be tested with each run.

Alcian Blue for PAS Staining Kit is tested upon manufacture to show that, when used in conjunction with the PAS Staining Kit, acid mucin stains bright blue, while neutral polysaccharides stain bright magenta, and nuclei stain light blue.

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.

LIMITATIONS

General Limitations

- 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.
- 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.
- The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, 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 positive and negative controls.

PERFORMANCE CHARACTERISTICS

BenchMark Special Stains Automated Slide Stainer

- Instrument-to-Instrument:** 100 slides of colon tissue were tested across 5 different BenchMark Special Stains instruments with the Alcian Blue for PAS 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.
- Run-to-Run:** 100 slides of colon tissue were tested across 5 BenchMark Special Stains instruments on 5 non-consecutive days with the Alcian Blue for PAS Staining Kit. The slides were evaluated for staining with pass or fail criteria. The results demonstrated no significant difference in staining intensity among slides.
- Compatibility with Alcian Blue for PAS Staining Kit** was tested with other selected special stains reagents. No significant adverse chemical interactions were observed.

NexES Special Stains Automated Slide Stainer

1. Intra-run reproducibility of Alcian Blue for PAS Staining Kit was determined by staining slides from 6 blocks of Barrett's esophagus, 2 blocks of colon and 2 blocks of small intestine for a total of 180 slides. The slides were evaluated for staining performance; differentiation of the mucin types (overall coverage and quality of the stain) and counterstain with pass or fail criteria. The results demonstrated that 180/180 slides performed per the acceptance criteria.
2. Inter-run reproducibility of staining with Alcian Blue for PAS Staining Kit was determined by staining 20 slides per run across 3 instruments (1 run per instrument). The slides were evaluated for staining performance, differentiation of the mucin types and counterstain with pass or fail criteria. The results demonstrated no significant difference in staining intensity among 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 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.

REFERENCES

1. Hotchkiss RD. A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch Biochem. 1948; 16:131-141.
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4. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.

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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.ventana.com



Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany

