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## INTENDED USE

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Periodic Acid Schiff (PAS) reagents are for "In Vitro Diagnostic Use".

Sigma-Aldrich PAS staining procedure offers standard and microwave procedures for the demonstration of lymphocytes and mucopolysaccharides. The staining pattern of the lymphocytes are helpful in making therapeutic decisions in established cases of lymphocytic leukemia. The PAS reaction in tissue sections is useful for the demonstration of mucopolysaccharides. The Diastase ( $\alpha$ -amylase) digestion procedure, followed by the PAS stain is useful as an aid in the diagnosis of glycogen storage disease.

The PAS staining procedure may also be used for the demonstration of fungal organisms in tissue sections.<sup>2</sup>

When treated with periodic acid, glycols are oxidized to aldehydes. After reaction with Schiff's reagent (a mixture of pararosaniline and sodium metabisulfite), a pararosaniline adduct is released that stains the glycol-containing cellular components.<sup>1</sup> This reaction can be performed on blood or bone marrow films, tissue touch preparations or tissue sections.<sup>2,3</sup> When used on blood or bone marrow films, this test may be helpful in recognizing some cases of erythroleukemia and acute lymphoblastic leukemia.<sup>4</sup>

Diastase ( $\alpha$ -amylase) digestion may be employed as an aid in the diagnosis of glycogen storage disease. Diastase hydrolyzes starch, glycogen and degradation products originating in these polysaccharides present in tissue. The resultant by-products of the digestion process are rinsed away prior to PAS staining.<sup>5</sup>

Sigma-Aldrich procedures include PAS techniques for rapid staining in microwave ovens.<sup>6-8</sup>

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## REAGENTS

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### PERIODIC ACID SOLUTION, Catalog No. 395-1

Periodic acid, 1 g/dl.

### SCHIFF'S REAGENT, Catalog No. 395-2

Pararosaniline HCl, 1%, and sodium metabisulfite, 4%, in hydrochloric acid, 0.25 mol/l.

### HEMATOXYLIN SOLUTION, GILL NO. 3, Catalog No. GHS-3

Certified hematoxylin, 6 g/l, sodium iodate, 0.6 g/l, aluminum sulfate, 52.8 g/l and stabilizer.

### STORAGE AND STABILITY:

Store Periodic Acid Solution, and Schiff's Reagent in refrigerator (2–8°C). Store Hematoxylin Solution, Gill No. 3 at room temperature (18–26°C). Reagents are stable until expiration date shown on labels. Formation of a precipitate in Schiff's Reagent, Catalog No. 395-2 does not affect performance.

### DETERIORATION:

Discard Hematoxylin Solution, Gill No. 3, if solution turns brown (over oxidized from air) or purple (loss of acidity) or when time required for suitable staining exceeds the time recommended in the procedure by more than 5 minutes.

### PREPARATION:

Periodic Acid Solution, Schiff's Reagent and Hematoxylin Solution, Gill No. 3 are supplied ready to use.

FORMALIN-ETHANOL FIXATIVE SOLUTION is prepared by mixing 5 ml of formaldehyde with 45 ml of 95% ethanol (Reagent Alcohol). Prepare fresh daily and keep tightly capped.

### PRECAUTIONS:

Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

PAS TISSUE-TROL control slides are paraffin embedded human tissue containing PAS and should be considered potentially infectious.

### US Risks and Safety Statements

Periodic Acid Solution is CORROSIVE. Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Take off immediately all contaminated clothing. Wear suitable protective clothing, gloves and eye/face protection.

Schiff's Reagent is TOXIC. Harmful if swallowed. Causes burns. May cause cancer. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Restricted to professional users. Avoid exposure - obtain special instructions before use.

Gill's 3 Hematoxylin solution is HARMFUL. Very toxic by inhalation. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Target organs: Liver and kidneys.

Reagent Alcohol is FLAMMABLE and an IRRITANT. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Xylene is FLAMMABLE and HARMFUL. Possible risk of impaired fertility. May cause harm to the unborn child. Harmful by inhalation and in contact with skin. Irritating to respiratory system and skin. Risk of serious damage to eyes. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

### EU Risks and Safety Statements

Periodic Acid Solution is CORROSIVE. Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Take off immediately all contaminated clothing. Wear suitable protective clothing, gloves and eye/face protection.

Schiff's Reagent is TOXIC. Causes burns. May cause cancer. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Restricted to professional users. Avoid exposure - obtain special instructions before use.

Gill's 3 Hematoxylin solution is HARMFUL. Very toxic by inhalation. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Reagent Alcohol is HIGHLY FLAMMABLE and an IRRITANT. Highly flammable. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Xylene is and HARMFUL. Flammable. Harmful by inhalation and in contact with skin. Irritating to skin. Avoid contact with eyes.

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## PROCEDURE

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### SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Freshly prepared whole, EDTA or heparinized blood or bone marrow films are used. Fix as soon as possible.<sup>4</sup>

For polysaccharides, tissue fixed in 10% neutral buffered formalin, Zenker's or Bouin's may be used.<sup>2</sup> It should be noted that some carbohydrates are water soluble.<sup>2</sup> For the demonstration of glycogen, Carnoy's fluid, Gendry's fluid or acid alcoholic formalin are recommended.<sup>2</sup> Time required for diastase extraction may be prolonged when tissue is fixed in a picric acid containing fixative.<sup>2</sup> Cut tissue sections at 5 microns.

### SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Formaldehyde solution, 37%

Reagent Alcohol

Whatman No. 4 filter paper

PAS control slides, such as Sigma PAS TISSUE-TROL, Catalog No. P8814, should be included in each run

### MICROWAVE PROCEDURES ONLY:

ACCUMATE™ H2100 Microwave Oven, Catalog Nos. A 9084 (110 v) or A 9209 (220 v)

Coplin jar with vented lids

Scott's Tap Water Substitute Concentrate, Catalog No. S5134-6x100ML

$\alpha$ -Amylase (for Diastase Extraction Procedure only)

### NOTES:

If the Sigma-Aldrich H2100 Microwave Oven is used, please see the Owner's Manual for instructions.

Blood films prepared from clinically healthy individuals may be included for control purposes. Polymorphonuclear leukocytes will show an intense red cytoplasmic stain. Tissue sections known to be PAS positive and/or contain glycogen should be included each time a stain sequence is performed. Sigma-Aldrich offers PAS TISSUE-TROL™, Catalog No. P 8814 for this purpose.

The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnostic tests or information.

### PROCEDURE:

#### I. BLOOD, BONE MARROW, OR TISSUE TOUCH PREPARATIONS

Standard Procedure:

1. Fix air dried blood films for **1 minute** at **room temperature** in Formalin-Ethanol Fixative Solution.
2. Rinse slides **1 minute** in slowly running tap water.
3. Immerse slides in Periodic Acid Solution, Catalog No. 395-1, for **5 minutes** at **room temperature**.
4. Rinse slides in several changes of distilled water.
5. Immerse slides in Schiff's Reagent, Catalog No. 395-2, for **15 minutes** at **room temperature**.  
NOTE: Immediately after use, cap Schiff's Reagent and return to refrigerator (2–8°C).
6. Wash slides in running tap water for **5 minutes**.
7. Counterstain slides in Hematoxylin Solution, Gill No. 3, Catalog No. GHS-3, for **90 seconds**.
8. Rinse slides in running tap water for **15–30 seconds**, air dry and examine microscopically under oil immersion (900x) lens. Slides may be mounted in toluene or xylene based mounting media.

#### Microwave Procedure:

1. Fix air dried films at **room temperature** for **1 minute** in Formalin-Ethanol Fixative.
2. Rinse slides for **1 minute** in slowly running tap water.
3. Place slides in **40 ml** of Periodic Acid Solution contained in a plastic Coplin jar.
4. Microwave on **800 watts** for **10 seconds**.
5. Rinse well in several changes of deionized water.
6. Place slides in **40 ml** Schiff's Reagent contained in a plastic Coplin jar.
7. Microwave at **800 watts** for **15 seconds**. Mix solution with a beral pipet or applicator stick and let incubate for **1 minute**.
8. Rinse in warm, gently running tap water for **5 minutes**.

9. Place slides in **40 ml** of Hematoxylin Solution Gill No. 3 contained in a plastic Coplin jar.
10. Microwave on **800 watts** for **10 seconds**.
11. Rinse in running tap water for **1–2 minutes**, then Blue in Working Scott's Tap Water Substitute at **room temperature**.
12. Rinse in running tap water. Air dry.
13. Slides may be mounted in toluene or xylene based mounting media.

## II. TISSUE SECTIONS

### Standard Procedure:

1. Deparaffinize and hydrate sections to deionized water.
2. Immerse slides in Periodic Acid Solution, Catalog No. 395-1, for **5 minutes** at **room temperature** (18–26°C).
3. Rinse slide in several changes of distilled water.
4. Immerse slides in Schiff's Reagent, Catalog No. 395-2, for **15 minutes** at **room temperature** (18–26°C).
5. Wash slides in running tap water for **5 minutes**.
6. Counterstain slides in Hematoxylin Solution, Gill No. 3, Catalog No. GHS-3, for **90 seconds**.
7. Rinse slides in running tap water.
8. Dehydrate, clear and mount sections in toluene or xylene based mounting media.

### Microwave Procedure for Diastase ( $\alpha$ -Amylase) Digestion:

1. Use duplicate test slides. Label one for digestion with diastase and one for PAS staining only.  
NOTE: Slides coated with a tissue adhesive are recommended.  
Do not celloidinize sections when doing diastase digestion.<sup>2</sup>
2. Deparaffinize and hydrate slides to deionized water.
3. Prepare Diastase ( $\alpha$ -amylase) Working Solution by dissolving **0.2 g  $\alpha$ -Amylase**, Catalog No. A 3176, to **40 ml** of deionized water. Mix well and place in plastic Coplin jar. Prepare just prior to use.
4. Microwave at **600 watts** for **25 seconds**.
5. Remove slides from Coplin jar and rinse digested slide in running tap water for **5 minutes**.
6. Using both the digested and the undigested slides, proceed with the microwave procedure for tissue, step 2.

### Microwave Procedure:

1. Deparaffinize and hydrate to deionized water.
2. Place slides in **40 ml** of Periodic Acid Solution contained in a plastic Coplin jar. Loosely cover jar with lid, or use lids with holes drilled into them.
3. Microwave at **800 watts** for **10 seconds**.
4. Rinse well in several changes of deionized water.
5. Place slides in **40 ml** Schiff's Reagent contained in a plastic Coplin jar.
6. Microwave at **800 watts** for **15 seconds**. Mix solution with a beral pipet or applicator stick and let incubate for **1 minute**.
7. Rinse in warm, gently running tap water for **5 minutes**.
8. Place slides in Hematoxylin, Gill No. 3, or Light Green Solution contained in a plastic Coplin jar.
9. Microwave on **800 watts** for **10 seconds**.
10. a. If using Gill's Hematoxylin, rinse in running tap water for **1–2 minutes**, then Blue in Working Scott's Tap Water Substitute at **room temperature**. Rinse in running tap water. Dehydrate, clear and mount.  
b. If using Light Green Solution, rinse quickly in deionized water, rapidly dehydrate, clear and mount.

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## PERFORMANCE CHARACTERISTICS

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PAS positive substances stain pink to red and nuclei are blue. A Diastase ( $\alpha$ -Amylase) Extraction slide will have no visible PAS staining of glycogen when compared to the undigested glycogen positive control slide.

If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

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## REFERENCES

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Procedure No. 395

Previous Revision: 2003-09

Revised: 2011-03



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