# Schiff reagent

CE IVD

**REF.320680**

Multi-application microscopy reagent

**IFU123A**

For professional use only.
Please read all information carefully before using this device.
IFU content may change, make sure you have the latest version available at my.ral-diagnostics.fr.

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## Intended use

Schiff reagent is intended to be used as multi-application microscopy reagent particularly in histo-cytological application prior microscopic examination.

If applicable, CellaVision RAL Diagnostics recommends using the associated CellaVision RAL Diagnostics products and cannot guarantee that the expected results will be achieved if used in combination with products of other brands.

## Principle

Schiff reagent in combination with other staining devices allows histo-cytology and mycology staining including Periodic Acid Schiff (PAS) and Feulgen staining.
PAS stains carbohydrates and glycoproteins in the wall of some pathogenic factors and helps to detect some parasitic and fungal elements. Its combine periodic acid as oxidizing agent, that breaks the bonds between two carbons of some chemical groups and Schiff reagent that stain released aldehydes functions. PAS can be applied on smears (cutaneous squama, oropharynx and esophagus cells, broncho-alveolar lavage fluid, smears through affixing of organs), or on histological sections.
Schiff reagent is also used for DNA assay in histo-cytological samples by two successive steps partial hydrolysis by HCl removing the purine bases (adenine and guanine), then this apuric DNA is stained by Schiff reagent.

## Device description

**Schiff reagent**
Clear colorless solution

| | |
|---|---|
| REF. 320680-0500 | 1 X 0.5 L |
| REF. 320680-1000 | 1 X 1 L |

For a specific batch, refer to the analysis certificate of the batch available at my.ral-diagnostics.fr.

## Storage and use conditions

Storage and use temperature: 15-25°C.
Storage and use conditions: away from light and heat sources.
Bottle shelf life before opening: refer to expiry date on the label.
Bottle shelf life after opening : refer to expiry date on the label and if the "period after opening" symbol is present take it into account.


## Hazard classification and safety information

**Schiff reagent**

Warning:
H319 - Causes serious eye irritation.
P280 - Wear protective gloves, protective clothing, eye protection.
P337+P313 - If eye irritation persists: Get medical advice/attention.

## Personnel qualification

All samples and products must be handled by qualified and authorized personnel, using individual or collective protection, in accordance with the national directives in force in the laboratories. Personnel must also be aware of the classification of hazardous materials indicated on the label and the safety data sheet (available at my.ral-diagnostics.fr).

The diagnosis must be conducted by qualified and authorized personnel, in accordance with the procedures in force within the laboratory.

## Specific equipment and reagents required but not provided

Hydrochloric acid, potassium metabisulphite, periodic acid, acetic acid, absolute ethanol, acetone, ether, sodium metabisulfite, lithium carbonate, microscope slides and these following RAL Diagnostic reagents:
Mayer Haemalum REF. 320550
Saffron in alcoholic solution REF. 369200

This equipment may vary depending on the protocol. Please refer to the relevant protocol (see the section operating procedure) to ensure that you have the necessary equipment to carry out tests.

## Operating procedure

The equipment used for sample processing must comply with the supplier's instructions for use.

**Sample preparation**
The specimen must be treated in accordance with procedures available in the laboratory and required by national authorities.

Histological sections: dewax and et hydrate tissues sections in appropriate reagents before staining.

**Reagents and instruments preparation**
The Schiff reagent is ready to use.
Sulphureous acid: Prepare extemporaneously 5 ml of HCl N + 5 ml of 10%-Potassium Metabisulphite + 100 ml of distilled water.
Periodic acid solution: prepare between 0.5 and 0.8% periodic acid solution.

**Protocols**
The staining steps of the protocols indicated below consist of a successive covering of the slides with the different staining reagents or dipping of the slides in the different staining baths. Please refer to the title to know which case you are in. For the covering method, place slide on a stand with fixed smear on top. The processing time only considers the dipping time in the reagents.

***Protocol for Feulgen Staining- Bath staining method - Manual microscopic analysis***

Processing time: 01:46:00

| Steps | Reagent | Time [mm: ss] | Indications |
|---|---|---|---|
| Rinse | HCl 1N solution | 01 :00 | Optional |
| Hydrolyze | HCl 1N solution | 05 :00 | Can be extended to 20 min according to fixative* |
| Stop hydrolyzing | Cold distilled water | NA | Stop hydrolyzing rinsing brusquely with cold distilled water |
| Stain | Schiff reagent | 90:00 | Can be extended to 2 hours then dry |
| Discolor | Sulphureous acid | 15:00 | Dip in 3 successive baths of 5 minutes in each |
| Rinse | Distilled water | NA | NA |
| Dehydrate | Croissant degrees ethanol | NA | To absolute ethanol |
| Dehydrate | Xylene or toluene | NA | Pass slide in |
| Mount | Xylene or toluene mounting media | NA | NA |

***Note:*** *Tissues sections are preferably fixed in Zenker, Helly, Baker, Carnoy, sublimate formalin or sublimate ethanol.*
** See underlying table.*

***Specificity Control:*** *Carry out the same reaction on another section cancelling hydrolysis (steps 1 to 3, Rinse, hydrolyze and stop hydrolyzing): DNA should not be colored.*

| Fixative | Time in min | Fixative | Time in min |
|---|---|---|---|
| Bouin-Allen | 22 | Helly | 8 |
| Sublimate Bouin-Allen | 14 | Stowell | 20 |
| Carnoy | 8 | Flemming | 16 |
| Formalin | 8 | Champy | 25 |
| Sublimate Formalin | 8 | Susa | 18 |
| Absolute ethanol | 5 | Regaud | 14 |
| Acetic Formalin Alcohol | 7 | Newcomer | 20 |
| Zenker | 5 | | |

***Table of hydrolysis time at 60°C in HCl N according to the fixative***

### *Protocol for periodic acid Schiff staining in Mycology and Parasitology - Manual covering method - Manual microscopic analysis*

Processing time: 02:00:30

| Steps | Reagent | Time [mm: ss] | Indications |
|---|---|---|---|
| Fix | 30% acetic acid aqueous solution | NA | Dry heating it very gently until total evaporation of the solution |
| Fix | 50/50 mixture of ethanol and acetone | NA | Dry until total evaporation of the solution, repeat 3 times |
| Fix | Ether | NA | Dry until total evaporation of the solution, repeat 2 times |
| Fix | 70° ethanol | 15:00 | Can be extended to 30 min |
| Fix | 95° ethanol | 15:00 | Pass in a bath |
| Rinse | Running water | NA | Running water |
| Pre-stain | Periodic acid | 10:00 | NA |
| Rinse | Running water | NA | NA |
| Stain | Schiff reagent | 16:00 | NA |
| Develop stain | Sodium metabisulfite | 06:00 | Without rinsing previous reagent |
| Rinse | Running water | 05:00 | NA |
| Stain | Mayer haemalum | 02:30 | NA |
| Rinse | Running water | No | Until turn in color |
| Differentiate | Lithium carbonate | 15:00 | saturated and filtered aqueous solution |
| Rinse | Running water | NA | NA |
| Rinse | Absolute ethanol | NA | NA |
| Stain | Saffron in alcoholic solution | 01: 00 | NA |
| Rinse | Absolute ethanol | 20: 00 | 2 x 10 min<br>Can be extend to 30 min (3 x 10 min) |
| Dehydrate | Xylene or toluene | 15: 00 | NA |
| Mount | Xylene or toluene mounting media | NA | NA |

### *Protocol for periodic acid Schiff staining of tissues sections in Histology - Manual bath method - Manual microscopic analysis*

Processing time: 00:39:00

| Steps | Reagent | Time [mm: ss] | Indications |
|---|---|---|---|
| Pre-stain | Periodic acid | 05:00 | Precise time requirement |
| Rinse | Running water | 05:00 | Can be extended to 10 min |
| Rinse | Distilled water | 02:00 | Can be extended to 3 min |
| Stain | Schiff reagent | 15:00 | Can be extended to 30 min |
| Rinse | Distilled water | 02:00 | Can be extended to 3 min |
| Rinse | Running water | 02:00 | Can be extended to 5 min |
| Stain | Mayer haemalum | 05:00 | NA |
| Rinse | Running water | 03: 00 | Rinse and let in a bath of running water for 3 to 5 min |
| Dehydrate | Croissant degrees ethanol | NA | To absolute ethanol |
| Dehydrate | Xylene or toluene | NA | Pass slide in |
| Mount | Xylene or toluene mounting media | NA | NA |

## Expected results

### *Feulgen staining*

**DNA:** pink-red

### *P.A.S. staining in Mycology and Parasitology*

P.A.S. reaction stains red more or less bright:

- the wall of fungal mycelian and levuriform elements
- the precystic and cystic forms of *Pneumocystis Carinii*
- some protozoa: trophozoites of *Entamoeba histolytica*, as well as the forepart of the *microsporidia* spore filament

Concerning metazoa, P.A.S. reaction eases the detection and the characterization of the cuticular (or lamellar) membrane of vesicular larva of *Ecchinococcus granulosus* (hydatidosis) and *Ecchinococcus multicularis* (alveolar echinococcosis).
P.A.S. reaction is also very useful to the identification of the cuticle of tissular nematoda (e.g., *Anisakis* larva) and of arthopides integumentary structures (*Demodex*, *Sarcoptes*, cutaneous mites causing myasis; *Tunga penetrans* contributing to sarcopsyllosis or similar organisms, pentastomes).

### *P.A.S. staining (Periodic Acid Schiff) of tissue sections in Histology*

**Nuclei**: blue
**Glucidic substances**: bright red
Main substances colored by Periodic Acid Schiff are:

- Polysaccharides: Glycogen, starch, cellulose, dextrans.
- Glycoproteins: Fucomucins of mucus cells, glycoproteins of basal cells, reticulin, collagen (slightly tinted), crystalline lens, bacterial capsules.
- Acid mucopolysaccharides: Connective fundamental substance (slightly colored), mucus cells (neither hyaluronic acids, nor chondrotin acids are stained, except some slightly sulphated).

If observed results vary from those expected, please contact CellaVision RAL Diagnostics technical service through your usual supplier for assistance.

## Performance

This medical device is state of the art. Its analytical performance, scientific validity and medical relevance are assessed in the CE marking review.

To ensure product performance, use clean and dry laboratory equipment.

The laboratory is responsible for notifying the manufacturer and state competent authority of any serious incident relating to the medical device uses.

## User quality control

Users are responsible for determining the appropriate quality control procedures for their laboratory and complying with applicable laboratory regulations.

These quality control procedures should only be performed by qualified personnel.

## Other products

For more information, please contact your usual supplier.

## Recommendations, notes and troubleshooting

**Products appearance**
If the appearance of the products differs from the description above, do not use it and contact CellaVision RAL Diagnostics technical service through your usual supplier for assistance.

**Procedure notes**
To prevent products degradation, please comply with the storage and handling recommendations specified in this manual.

In the case of Feulgen staining, to assert the positivity of the reaction, Schiff's reagent must stain the sample only after lysis and does not stain the same unlysed sample.

**Product stability**
Every CellaVision RAL Diagnostics product can be used until the expiry date indicated on, in its original packaging if it is still hermetically sealed.

**Staining stability**
Staining quality and reproducibility depend on the correct use of the products. Staining conducted according to these recommendations will remain stable for several days. If it is necessary to store the stained smears for several months or years, CellaVision RAL Diagnostics recommended mounting them with a coverslip, using a suitable mounting liquid and storing them in a light and dustproof container.

**Instructions for cleaning and waste disposal**
All biological samples, effluents and used consumables should be considered potentially hazardous.

To avoid any risk, apply the following instructions: dispose of samples, effluents and consumables in accordance with laboratory standards and applicable national and local standards and regulations.

Chemical and biological waste must be collected and processed by specialized, registered companies.

## Table of symbols and abbreviations

Depending on the product, you may find the following symbols on the device or the packaging material.

| GHS Pictograms | Interpretation |
|---|---|
|  | Explosive |
|  | Flammable |
|  | Oxidizer |
|  | Compresses gas |
|  | Corrosive |
|  | Toxic |
|  | Harmful |
|  | Health Hazard |
|  | Environmental Hazard |
|  | No labelling applicable |

| Symbols | Interpretation |
|---|---|
|  | Batch code |
|  | Serial number |
|  | Catalogue reference |
|  | Date of manufacture |
|  | Use up to |
|  | Unique device identifier |
|  | Manufacturer |
|  | Importer |
|  | Entity distributing the medical advice in the region concerned |
|  | CE marking device |
|  | In vitro diagnostic medical device |
|  | Authorised Representative in the European Community |
|  | Authorised Representative in Switzerland |
|  | Complies with UK guidelines |
|  | Do not use if packaging is damaged |
|  | Keep away from light |
|  | Temperature limit: 15-25°C |
|  | Temperature limit: 15-30°C |
|  | Keep dry |
|  | Box: handling upwards |
|  | Fragile |
|  | Sterilised by irradiation |
|  | Single sterile barrier system with outer protective packaging |
|  | Sterile and radiation-sterilised barrier suit |
|  | Do not reuse |
|  | Do not resterilize |
|  | Contents sufficient for n tests |
|  | Hazardous material contained |
|  | Consult instructions for use |
|  | Use |
|  | After opening, use within XX months |
|  | The product must not be used in conjunction with an automatic colouring machine |
|  | Indicates a medical device that contains potentially carcinogenic, mutagenic or reprotoxic (CMR) substances, or substances classified as endocrine disruptors |

## Bibliography

**GANTER P., JOLLES G.,** *Histochimie normale et pathologique,* ed. GAUTHIER-VILLARS, vol. 2, 1970, p. 1526-1527 and 1533-1534.

**SEGRETAIN G., DROUHET E., MARIAT F.,** *Diagnostic de laboratoire en mycologie médicale*, Maloine, 3ème éd., 1974, p. 125-126.

## Changes tracking

| Date | Version | Changes |
|---|---|---|
| 02/2023 | IFU123A | IVDR (EU) 2017/746 compliance |

## Legal representatives

| Country | Address |
|---|---|
| United Kingdom | QAvis UK Ltd, company N° SC679796, 56-66 Frederick Street Edinburgh, EH21LS, United Kingdom |
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