Mayer haemalum

REF. 320550

Nuclear dye for histo-cytology staining

CE IVD

IFU090A-RAL

For professional use only.
Please read all information carefully before using this device.

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Intended Use

Mayer haemalum is intended to be used in combination with other staining devices for differential staining of cellular structures prior microscopic examination.

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

Principle

Mayer haemalum in combination with different staining devices allows histo-cytological and hormone-cytological staining. The histo-cytology applications include Gram Weigert, Periodic Acid Schiff (PAS), staining with orcein, haemalum-eosin staining and haemalum-eosin-saffron staining.
Gram Weigert, Periodic Acid Schiff (PAS) stain carbohydrates and glycoproteins in tissues, in the wall of some pathogenic factors and helps to detect some parasitic and fungal elements.
Additional specific dyes allow staining of elastic fibers for staining with orcein.
Haemalum-eosin staining and its variations allow besides the progressive staining of the nucleus by the haemalum, to stain collagen, cytoplasm, elastic fibers and erythrocytes.
Haemalum-Shorr staining combines heamalum to Shorr solution to carry out the morphological study of spermatozoa called spermocytogram.

Device description

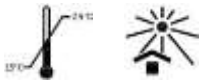
Mayer haemalum
Clear red violet solution

REF. 320550-1000	1 X 1.0 L
REF. 320550-2500	1 X 2.5 L

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

Storage

Storage temperature: 15-25°C away from light.
Bottle shelf life before and after opening: refer to expiry date on label.



Hazard classification and safety information

Mayer haemalum
Warning: H226 - Flammable liquid and vapour. H302 - Harmful if swallowed. H371 - May cause damage to organs.
P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P264 - Wash hands thoroughly after handling. P308+P311 - IF exposed or concerned: Call a POISON CENTER or doctor.

CONT	CH3OH

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

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

Sodium hyposulfite, aniline, iodin, acetone, glacial acetic acid, ethanol, lithium carbonate, periodic acid, ether, sodium metabisulfite, concentrated HCl, microscope slides, and these following RAL Diagnostics devices:
Carbolic gentian violet REF. 320960
Eosin, 1% in aqueous solution REF. 312740
Erythrosin 239, 1% in aqueous solution REF. 361820
Lugol solution REF. 367300
Orcein, synthetic solution REF. 315520
Phloxine B, 3% in aqueous solution REF. 350750
Shorr solution REF. 361100
Saffron in alcoholic solution REF. 369200
Schiff reagent REF. 320680

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

Specimen must treat in accordance with procedures available in the laboratory and promulgated by national authorities.
Histological sections: dewax and et hydrate tissues sections in appropriate reagents before staining.
Mycology and Parasitology: fix specimen before staining
Spermocytograms: made a smear

If the smear was fixed by cytofixer, remove cytofixer by dipping the slide in 50°-Alcohol for 20 minutes. If it's not, fix the smear in a 95°-Alcohol/ether bath (50/50) for 5 minutes.

Reagents and instruments preparation

Sodium hyposulfite solution: dissolve 3 g of sodium hyposulfite in 100 mL of distilled water.
Periodic acid solution: prepare between 0.5 and 0.8% periodic acid solution
Clarke fixative: mix75 mL 95° ethanol and 25 mL glacial acetic acid
Orcein Solution: dissolve 1 g of orcein in 100 mL of 70° ethanol and 0,6 mL of concentrated HCl.
Acid-alcohol Solution: put 1 mL of concentrated HCl in 100 mL of 70° ethanol
Iodined Ethanol solution: dissolve 0.5g of Iodin in 100 mL of 80° ethanol

Protocols

The staining steps of the protocols indicated below consist of a successive dipping of the slides in the different staining baths.

Protocol for Gram Weigert staining for histological sections - Manual covering method with Slow differentiator - Manual microscopic analysis

This method applies to cytology and histological sections as well as fixed with chromic fixative (Zenker, Helly) and embedded in paraffin.

Protocol to get rid of mercury precipitates
Mercury precipitates could bother the reading of the preparations, carry out after 95° ethanol bath during the dewaxing
Processing time: 03 min

Steps	Reagent	Time [mm: ss]	Indications
Clean	Iodined Ethanol solution	No	Dip the slide
Rinse	Tap water	No	Quickly
Clean	3% Sodium Hyposulfite solution	03:00	Dip the slide
Rinse	Tap water	No	Rinse well
Rinse	Distilled water	No	No

Staining protocol
Processing time: 17 min

Steps	Reagent	Time [mm: ss]	Indications
Stain	Mayer haemalum	03:00	Can be extended to 5 min
Rinse	Running water	03:00	Let it in a bath of running water. Can be extended to 5 min.
Differentiate	hydrochloric alcohol or lithium carbonate	No	If necessary to get a clear nuclear staining
Stain	Eosin, 1% in aqueous	01:00	Can be extended to 5 min
Rinse	Running water	No	No
Stain	Carbolic Gentian Violet	05:00	Can be extended to 10 min
Rinse	Water	No	Quickly
Fix dye	Lugol solution	05:00	No
Rinse	Water	No	Quickly Drain on filter paper to get rid of reagent the water excess
Differentiate and dehydrate	50/50 mixture of aniline and xylene or toluene	No	No
Dehydrate	Xylene or toluene	No	Pass slide in
Mount	Xylene or toluene mounting media	No	No

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

Processing time: 39 min

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	No
Rinse	Running water	03: 00	Rinse and let in a bath of running water for 3 to 5 min
Dehydrate	Croissant degrees ethanol	No	To absolute ethanol
Dehydrate	Xylene or toluene	No	Pass slide in
Mount	Xylene or toluene mounting media	No	No

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

Processing time: 120 min 30 s

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

Protocol for heamalum- eosin staining of tissues sections - Manual bath method - Manual microscopic analysis

Processing time: 11 min

Steps	Reagent	Time [mm: ss]	Indications
Stain	Mayer haemalum	03:00	Can be extended to 5 min
Rinse	Running water	03:00	Can be extended to 5 min
Stain	Eosin, 1% in aqueous solution	05:00	Can be extended to 7 min
Rinse	Running water	No	No
Dehydrate	Croissant degrees ethanol	No	To absolute ethanol
Dehydrate	Xylene or toluene	No	Pass slide in
Mount	Xylene or toluene mounting media	No	No

Protocol for heamalum- eosin variant with Phyloxine B, staining of tissues sections - Manual bath method - Manual microscopic analysis

Processing time: 08 min

Steps	Reagent	Time [mm: ss]	Indications
Stain	Mayer haemalum	03:00	Can be extended to 5 min
Rinse	Running water	03:00	Can be extended to 5 min
Stain	Phyloxine B, 3 % in aqueous solution	02:00	Can be extended
Rinse	Running water	No	No
Dehydrate	Croissant degrees ethanol	No	To absolute ethanol
Dehydrate	Xylene or toluene	No	Pass slide in
Mount	Xylene or toluene mounting media	No	No

Protocol for heamalum- eosin variant with Erythrosin 239, 1%, staining of tissues sections - Manual bath method - Manual microscopic analysis

Processing time: 11 min

Steps	Reagent	Time [mm: ss]	Indications
Stain	Mayer haemalum	03:00	Can be extended to 5 min
Rinse	Running water	03:00	Can be extended to 5 min
Stain	Erythrosin 239, 1% in aqueous solution	05:00	No
Rinse	Running water	No	No
Dehydrate	Croissant degrees ethanol	No	To absolute ethanol
Dehydrate	Xylene or toluene	No	Pass slide in
Mount	Xylene or toluene mounting media	No	No

Protocol for heamalum- eosin saffron staining of tissues sections - Manual bath method - Manual microscopic analysis

Processing time: 16 min

Steps	Reagent	Time [mm: ss]	Indications
Stain	Mayer haemalum	03:00	Can be extended to 5 min
Rinse	Running water	03:00	Can be extended to 5 min
Stain	Eosin, 1% in aqueous solution	05:00	Can be extended to 7 min
Rinse	Running water	No	No
Dehydrate	Croissant degrees ethanol	No	To absolute ethanol
Stain	Saffron in alcoholic solution	05:00	Can be extended to 8 min
Rinse	Absolute ethanol	No	Quickly
Dehydrate	Xylene or toluene	No	Pass slide in
Mount	Xylene or toluene mounting media	No	No

Protocol for heamalum- eosin saffron variant with Phyloxine B, staining of tissues sections - Manual bath method - Manual microscopic analysis

Processing time: 13 min

Steps	Reagent	Time [mm: ss]	Indications
Stain	Mayer haemalum	03:00	Can be extended to 5 min
Rinse	Running water	03:00	Can be extended to 5 min
Stain	Phyloxine B, 3 % in aqueous solution	02:00	Can be extended
Rinse	Running water	No	No
Dehydrate	Croissant degrees ethanol	No	To absolute ethanol
Stain	Saffron in alcoholic solution	05:00	Can be extended to 8 min
Rinse	Absolute ethanol	No	Quickly
Dehydrate	Xylene or toluene	No	Pass slide in
Mount	Xylene or toluene mounting media	No	No

Protocol for heamalum- eosin-saffron variant with Erythrosin 239, 1%, staining of tissues sections - Manual bath method - Manual microscopic analysis

Processing time: 16 min

Steps	Reagent	Time [mm: ss]	Indications
Stain	Mayer haemalum	03:00	Can be extended to 5 min
Rinse	Running water	03:00	Can be extended to 5 min Let in bath of running water
Stain	Erythrosin 239, 1% in aqueous solution	05:00	No
Rinse	Running water	No	No
Dehydrate	Croissant degrees ethanol	No	To absolute ethanol
Stain	Saffron in alcoholic solution	05:00	Can be extended to 8 min
Rinse	Absolute ethanol	No	Quickly
Dehydrate	Xylene or toluene	No	Pass slide in
Mount	Xylene or toluene mounting media	No	No

Protocol for staining of tissues sections with orcein- Manual bath method - Manual microscopic analysis

Processing time: 47 min

Steps	Reagent	Time [mm: ss]	Indications
Stain	Orcein	30: 00	Can be extended to 60 min
Rinse	Distilled water	No	Drain on filter paper the water excess
Clean	95° ethanol	No	remove the excess stain
Differentiate	absolute ethanol	05:00	Can be extended up to 30 min, until the section stains pale brown*
Discolor	Acid-alcohol Solution	02:00	Discolor background until quite colorless. Can be extended to 10 min**
Rinse	Running water	05:00	At least
Stain	Mayer haemalum	05:00	No
Dehydrate	95° ethanol	No	No
Dehydrate	Absolute ethanol	No	2 baths
Dehydrate	Xylene or toluene	No	2 baths
Mount	Xylene or toluene mounting media	No	No

* Check under the microscope whether the elastic fibers are black indeed
** Check under the microscope.

Protocol for spermocytograms haemalum-Shorr staining - Manual bath method - Manual microscopic analysis

Processing time: 23 min 15 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	Clarke Fixative	00: 15	No
Stain	Mayer haemalum	08: 00	No
Differentiate	alkaline water or running water	05: 00	essential to differentiate the. Drain on filter paper to get rid of reagent the water excess
Rinse	70° ethanol	No	Pass in a bath
Stain	Shorr Solution	10: 00	No
Dehydrate	95° ethanol	No	Pass in a bath
Dehydrate	Absolute ethanol	No	2 baths
Dehydrate	Toluene or xylene	No	No
Mount	Toluene or Xylene based mounting medium	No	No

Expected results

Gram Weigert staining
Nuclei: blue to blackish blue
Bacteria Wall (Pneumocystis carinii, mycosis): violet

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).

P.A.S. Staining of Smears 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).

Haemalum-eosin staining and its variations
Nuclei: blue to blackish blue
Collagen: very Pale Pink
Cytoplasm: pink to Red
Elastic Fibers: light Pink
Erythrocytes: light Pink

Haemalum-eosin-saffron staining and its variations
Nuclei: blue to blackish blue
Collagen: golden yellow to ocre (mucus, ground substance of cartilage or of bone are equally colored yellow)
Cytoplasm: pink
Elastic fibers: pink
Erythrocytes: light pink

Staining with Orcein
Elastic fibers: blackish Brown
Nuclei: black

Haemalum-Shorr Staining for Spermocytograms
Head piece -Nucleus: purple
Head piece-Acrosome: green blue
Flagellum: green
Midpiece: pale green
During the analysis, Assess in percentage:

- abnormalities of the head, midpiece and flagellum
- agglutinates
- leukocytes, erythrocytes, cells

If observed results vary from those expected, please contact 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.

RAL Diagnostics recommends quality control at reagents renewal and for the first staining cycle of each day. Slides stained for quality control purposes should be checked to ensure that they are satisfactory for intended test (properly stained and free of precipitate). Staining results for each cell type must also be compliant with this manual expected results.

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

Other products

For more information 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 RAL Diagnostics technical service through your usual supplier for assistance.

Procedures notes

To prevent products degradation, please comply with the storage and handling recommendations specified in this manual.
Filter Mayer heamalun solution before each use.
Staining times may vary according to the type and thickness of tissues

Products stability

Every 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.
RAL Diagnostics recommends mounting the stained slides with a coverslip using a suitable mounting liquid and to store 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

SYMBOL	INTERPRETATION
LOT	Batch code
SN	Serial number
REF	Catalogue reference
	Date of manufacture
	Use up to
UDI	Unique device identifier
	Manufacturer
	Importer
	Entity distributing the medical advice in the region concerned
CE	CE marking device
IVD	In vitro diagnostic medical device
	Authorised Representative in the European Community
	Authorised Representative in Switzerland
UK CA	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	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

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Change tracking

Date	Version	Changes
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