Carbolic solution

REF. 320790-0500

Diluent for thiazine red solution

CE IVD

IFU074A-RAL

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

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Intended use

Carbolic solution in combination with Carbolic auramine, Degommier discolouring solution and Thiazine red concentrated solution is intended to be used for acid fast bacilli differential fluorescent staining 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

Carbolic solution in combination with Carbolic auramine, Degommier discolouring solution and Thiazine red concentrated solution allow a quick fluorescence detection of mycobacteria or Acid Fast Bacilli (AFB). The characteristic structure of the mycobacteria walls hampers discoloring agent penetration. This property allows AFB to keep auramine staining after discoloring with acid and alcohol. Other bacteria (non-AFB) and cell elements are counterstained by Thiazine Red.

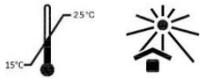
Device description

Carbolic solution
Clear colorless solution
REF. 320790-0500 1 X 500 mL

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 the expiry date on the label.
Once opened, the duration of use overrules the expiry date



Hazard classification and safety information

Carbolic Solution
No labelling applicable

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

Microscope slides, methanol, fluorescence microscope with FITC filter Set (Auramine: Ex. 430 nm/Em. 510 nm, Thiazine Red: Ex. 510 nm/ Em. 600 nm) and these following RAL Diagnostics devices:
Carbolic auramine REF. 361430
Degommier discolouring solution REF. 320800
Thiazine red concentrated solution REF. 320780
SUREFIX REF. 336000-0050

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
Pre-treatment of sample from liquid culture media: Take around 300 to 400 µL of liquid culture medium (including a few beads if possible) and pour it into an microtube. Centrifuge for 1 min at 10 000 rpm and discard supernatant. Then add 2 to 3 drops of physiological saline to the microtube and vortex or stir with a loop. The sample is now ready to be smeared.

Manual bacterial smear: made a thin film of bacteria sample and leave the slide to dry at room temperature. Then bacterial smear can be fixed with mild heat source (Bunsen burner or hot plate) or chemically fixed with chemical fixative (methanol, ethanol, acetic acid, or formalin…)
NB: Never pass through the flame a smear that is not entirely dry, this could cause cracklings and dissemination of bacteria (creation of aerosols).
If necessary, the two fixations can be combined.

Manual bacterial smear from liquid or solid culture: Apply a drop of SUREFIX on a slide and with a loop place on top of the SUREFIX drop, the preparation from a liquid culture (as described above) or a colony from a solid culture. Mix SUREFIX drop and the sample and made a uniform smear layer. Eventually Leave the smear to air dry before placing the slide on a hot plate for 30 min at a temperature of 80 °C.

Reagents and instruments preparation
Prepare the needed amount of thiazine red solution by mixing equal quantities of Carbolic Solution and Thiazine Red concentrate solution.

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

Standard protocol for bacterial smear staining (recommended)– manual bath method - Manual microscopic analysis

Processing time: 38 min

Steps	Reagent	Time [mm: ss]	Indications
Fix	Methanol	10: 00	No
Rinse	Distilled water	No	
Stain	Carbolic auramine	20: 00	
Rinse	Distilled water	No	
Discolor	Degommier discolouring solution	03: 00	
Rinse	Distilled water	No	
Stain	Thiazine Red solution	05: 00	
Rinse	Distilled water	No	
Dry	No	≥03:00	Keep away from light

Expected results

Bacterial smear
A.F.B: fluorescent green yellow
Background of the preparation: red

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 use of the medical device.

User quality Control

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

RAL Diagnostics recommend using a positive smear and a negative smear from different patient samples 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).
Slides can be prepared in advance and heat-fixed appropriately for storage.
This control could be done using a positive patient sample or a dilute suspension of AFB recognized positive (such as *Mycobacterium abscessus* CIP 108541).
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.

It is necessary to perform a preliminary fixation of the bacterial smear.
Air dry away from light.
The microscopic examination is usually performed with x20 and x40 dry objectives and without coverslip. Each time fluorescent organisms are observed, it is necessary to confirm the presence of Acid Fast Bacilli (AFB) by staining the slide anew with one Ziehl cold or hot staining technique (Kit RAL ZN staining or kit Cold ZN).

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.

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
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
EC REP	Authorised Representative in the European Community
CH REP	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
STERILE R	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
CONT	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

Bibliography

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GROSSET J., CATALAN F., LEVENTIS S., *Le problème actuel des mycobactéries*, Biologiste et Praticien, n°39, 1979, p. 1-22.
PACAUD G., *Coloration en mycobactériologie*, Réactifs RAL, 1977, p. 7-10.
PACAUD G., *Les colorations dans la pratique quotidienne en mycobactériologie*, ATEB, Journée Technique Parisienne, mars 1977.

Change tracking

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