RAL ZN staining kit (3 x 1 L)

REF. 365400-0000

Acid fast bacilli differential staining

CE IVD

IFU075A-RAL

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

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Intended use

RAL ZN staining kit is intended to be used for acid fast bacilli differential 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

Ziehl-Neelsen staining allows a 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 ZN Carbolic Fuchsin staining after discoloring with ZN Acid-Alcohol 3%. Other bacteria (non-AFB) and cell elements are counterstained by ZN Methylene Blue 0.3%.

Kit description

ZN Carbolic Fuchsin
Clear dark red solution
REF. 365310-1000 1 X 1 L

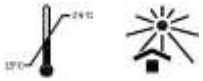
ZN Acid-Alcohol 3%
Clear colorless solution
REF. 365320-1000 1 X 1 L

ZN Methylene Blue 0.3%
Clear blue solution
REF. 365330-1000 1 X 1 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 opening: refer to the expiry date on the label.
Bottle shelf life after opening: 400 to 600 slides.
Once opened, the duration of use overrules the expiry date.



Hazard classification and safety information

ZN Carbolic Fuchsin

Danger: H226 - Flammable liquid and vapour. H302+H332 - Harmful if swallowed or if inhaled. H314 - Causes severe skin burns and eye damage. H341 - Suspected of causing genetic defects.
P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P261 - Avoid breathing dust/fume/gas/mist/vapours/spray. P280 - Wear protective gloves, protective clothing, eye protection, face protection. P303+P361+P353+P310 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. Immediately call a POISON CENTER or doctor. P305+P351+P338+P310 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor.

CONT	C6H5OH 80%

ZN Acid-Alcohol 3%

H225 - Highly flammable liquid and vapour. H314 - Causes severe skin burns and eye damage.
P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P280 - Wear protective gloves, protective clothing, eye protection. P301+P330+P331+P310 - IF SWALLOWED: rinse mouth. Do NOT induce vomiting. Immediately call a POISON CENTER or doctor. P303+P361+P353+P310 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. Immediately call a POISON CENTER or doctor. P305+P351+P338+P310 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor.

CONT	HCl 25%

ZN Methylene Blue 0.3%
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, hot plate or Bunsen burner or alcohol-burning cotton wool and following RAL Diagnostics device:
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
No preparation needed. The solutions are ready to use.

Protocols
The staining steps of the protocols indicated below consist of a successive covering of the slides with the different staining reagents.
For the staining steps, place slide on a stand with fixed smear on top.

Protocol for bacterial smear staining – Manual covering method - Manual microscopic analysis

Processing time: 09 min

Steps	Reagent	Time [mm: ss]	Indications
Stain	ZN Carbolic Fuchsin	No	Place the slide on hot plate fixed smear on top. Cover the smear with the reagent.
Heat	ZN Carbolic Fuchsin	05: 00	Add ZN Carbolic Fuchsin time to time to avoid desiccation. Be careful not to boil the ZN Carbolic Fuchsin
Rinse	Tap water	No	Rinse carefully, smear is red at this stage
Discolor	ZN Acid-Alcohol 3%	03: 00	red color should have almost disappeared. If not, repeat the ZN Acid-Alcohol 3% procedure for a further 2 minutes.
Rinse	Tap water	No	Rinse carefully, get rid of excess stain and remove excess of rinsing water
Stain	ZN Methylene Blue 0.3%	01: 00	No
Rinse	Tap water	No	No
Dry	No	≥03: 00	No

Expected results

Bacterial smear
A.F.B: pink
Background of the preparation: blue

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.
Microscopic examination is performed with objective X100 immersion.
According to the thickness of the smear, it may be necessary to increase the ZN Carbolic Fuchsin staining time.
To heat the slides, Bunsen burner, alcohol lamp, alcohol-burning cotton wool are also suitable.
To realize a screening of specimen samples, it's recommended to use an auramine fluorescence technique before. The observation of a single bacillus on a given slide is a dubious result and should always lead to a new investigation on another sample.
In all cases, the bacteriologist's report should always refer to the number of fields observed and be consequently reported as "no AFB detected on 200 (or 100) microscopic fields" and not as "negative bacilloscopy".
Likewise, "positive bacilloscopy" is also a bad answer because it gives no indication of the sputum relative richness in bacilli. The report must always provide quantitative information.

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. Staining conducted according to these recommendations will remain stable for several days.

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

Bibliography

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Change tracking

Date	Version	Changes
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