Kit Cold ZN

REF. 362390-0000

Acid fast bacilli differential staining

CE IVD

IFU078A-RAL

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

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Intended Use

Kit Cold ZN 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

Kit Cold ZN 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 Fuchsin staining after discoloring with acid and alcohol. Other bacteria (non-AFB) and cell elements are counterstained by Methylene Blue.

Kit description

Fixative
Clear colorless solution
REF. 362120-0240 1 X 240 mL

Fuchsin
Clear dark red solution
REF. 365250-0240 1 X 240 mL

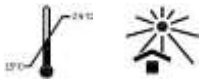
Discolouring Solution
Clear colorless solution
REF. 362215-0240 1 X 240 mL

Methylene Blue
Clear blue solution
REF. 365340-0240 1 X 240 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.



Active components

Fixative
Trichloroacetic acid -CAS- 76-03-9: ≤ 1%

Fuchsin
Ethanol -CAS- 64-17-5: < 20%
Basic fuchsin, diamant -CAS- 632-99-5: ≤ 3%
Phenol -CAS- 108-95-2: < 7%

Discolouring Solution
Ethanol -CAS- 64-17-5: ≤ 95 %
Hydrochloric acid, 37% -CAS- 7647-01-0: < 1%

Methylene Blue
Methylene blue -CAS- 61-73-4: < 0.5%

Hazard classification and safety information

Fixative
Danger: H314 - Causes severe skin burns and eye damage. H412 - Harmful to aquatic life with long lasting effects.
P280 - Wear protective clothing, protective gloves, 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.

Fuchsin

Danger: H226 - Flammable liquid and vapour. H302 - Harmful if swallowed. H314 - Causes severe skin burns and eye damage. H341 - Suspected of causing genetic defects. H351 - Suspected of causing cancer.
P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P264 - Wash hands thoroughly after handling. 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	Basic fuchsin, diamant, C6H5OH 80%

Discolouring Solution

Danger: 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.

Methylene Blue

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 and these following RAL Diagnostics devices:
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 or dipping of the slides in the different staining baths. The information is in the title of the protocols.

For the covering method, place slide on a stand with fixed smear on top.

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

Processing time: 18 min 30 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	Fixative	01: 00	No
Rinse	Water bath	00: 30	Drain reagent excess onto absorbent paper then rinse
Stain	Fuchsin	10: 00	No
Rinse	Water bath	01: 00	Drain reagent excess onto absorbent paper then rinse
Discolor	Discolouring Solution	03: 00	No
Rinse	Water bath	01: 00	Drain reagent excess onto absorbent paper then rinse
Stain	Methylene Blue	01: 00	No
Rinse	Water bath	01: 00	Drain reagent excess onto absorbent paper then rinse
Dry	No	≥03:00	No

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

Processing time: 18 min 30 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	Fixative	01: 00	No
Rinse	Water bath	00: 30	Ged rid of the reagent then rinse
Stain	Fuchsin	10: 00	No
Rinse	Water bath	01: 00	Ged rid of the reagent then rinse
Discolor	Discolouring Solution	03: 00	No
Rinse	Water bath	01: 00	Ged rid of the reagent then rinse
Stain	Methylene Blue	01: 00	No
Rinse	Water bath	01: 00	Ged rid of the reagent then rinse
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 Fuchsin staining time.
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.
It is recommended to use two different staining kits, one for direct examination smears and another one for smears coming from cultures.

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
05/2022	IFU078A-RAL	IVDR (EU) 2017/746 compliance

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