Kit Gram-Hücker L, slow action

REF.362860-0000

Differential staining of bacteria

CE IVD

IFU043A-RAL

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

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Intended Use

Kit Gram-Hücker L, slow action is intended to be used for differential staining of bacteria 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

Gram-Hücker staining is a differential staining based on the permeability of the bacterial wall. In this technique, the bacterial wall is not stained but its structure permits classification of Gram-positive or Gram-negative bacteria.
Lugol solution allows the formation of an intracellular complex with Crystal violet oxalate. A more important permeability of Gram-negative bacteria wall allows alcohol to eliminate this complex. Gram-negative bacteria can fix Safranin and then appear stained orangey-pink. Gram-positive bacteria, characterized by a less important permeability of wall, are not discolored by Alcohol and remain stained violet.
Gram-Hücker Staining, which original aim is to differentiate Gram-negative bacteria from Gram-positive ones, is very useful in Mycology and Parasitology (medical and veterinary). Indeed, pathogenic fungi are Gram-positive and this specific property can be very beneficial to detect this kind of agents on samples. Microsporidia spores are Gram-positive as well and can then be detected on smears, e.g. through affixing of duodenal biopsies.

Kit description

Crystal violet oxalate
Clear dark purple solution
REF. 3614901A240 1 X 240 mL

Lugol, PVP-stabilized solution
Clear brown solution
REF. 3674002A240 1 X 240 mL

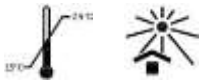
Slow differentiator (alcohol-based)
Clear colorless solution
REF. 363030-0240 1 X 240 mL

Safranin
Clear red solution
REF. 3615004A240 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 expiry date on label.



Hazard classification and safety information

Crystal violet oxalate

Warning: H226 - Flammable liquid and vapour. H319 - Causes serious eye irritation. H351 - Suspected of causing cancer. H412 - Harmful to aquatic life with long lasting effects.
P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P280 - Wear protective gloves, protective clothing, eye protection, face protection. P308+P313 - IF exposed or concerned: Get medical advice/attention. P337+P313 - If eye irritation persists: Get medical advice/attention.

CONT	Crystal violet

Lugol, PVP-stabilized solution
No labelling applicable

Slow differentiator (alcohol-based)

Danger: H225 - Highly flammable liquid and vapour. H318 - Causes serious 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. 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	CH3(CH2)3OH

Safranin

Warning: H226 - Flammable liquid and vapour.
P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

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, mild heat source (Bunsen burner or hot plate), chemical fixative (methanol, ethanol, acetic acid, or formalin etc.) 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.
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: 02 min 50 s

Steps	Reagent	Time [mm: ss]	Indications
Stain	Crystal violet oxalate	01:00	No
Rinse	Water	No	Get rid of reagent and remove the excess
Rinse	Lugol, PVP-stabilized solution	No	A jet of Lugol to remove rinsing water
Stain	Lugol, PVP-stabilized solution	00:30	Can be extended to 1 min
Rinse	Water	No	Thoroughly rinse
Discolor	Slow Differentiator	00:20	Can be extend to 40 sec
Rinse	Water	No	Quickly
Stain	Safranin	01:00	No
Rinse	Water	No	Quickly
Dry	No	≥03:00	No

Protocol for smear staining in Mycology and Parasitology - Manual covering method - Manual microscopic analysis

Processing time: 01 min 40 s

Steps	Reagent	Time [mm: ss]	Indications
Stain	Crystal violet oxalate	01:00	No
Rinse	Water	No	Get rid of reagent and remove the excess
Rinse	Lugol, PVP-stabilized solution	No	A jet of Lugol to remove rinsing water
Stain	Lugol, PVP-stabilized solution	00:30	Can be extended to 1 min
Rinse	Water	No	Thoroughly rinse
Discolor	Slow Differentiator	No	No
Rinse	Water	No	Quickly
Stain	Safranin	00:10	Can be extended to 1 min
Rinse	Water	No	Quickly
Dry	No	≥03:00	Quickly

Expected results

Bacterial smear

Gram-positive Bacteria: violet
Gram-negative Bacteria: orangey – pink

Parasitology and Mycology

Pathogenic fungi, Microsporidia spores: violet.

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

The following example is for bacterial samples.

RAL Diagnostics recommend using a Gram positive and a Gram negative sample for reagents quality control at reagents renewal, for each staining set or at least for the first staining cycle if a stain is performed multiple times daily.

These slides can be prepared in advance and heat-fixed appropriately for storage.
This control could be done using Gram positive and Gram negative samples from identified patient samples or using a known Gram positive and Gram negative strains (such as *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922). The strains used must be identified, avoid Gram variable species.
Staining results for each cell type must also be compliant with this manual expected results.

These quality controls depend on the authorization by qualified personnel.

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

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.

Adding of Polyvinylpyrrolidone (PVP) to Lugol, PVP-stabilized solution helps to avoid Iodine migration and then provides a satisfactory stability of the ready-to-use plastic bottle packaged solutions.
One can improve the sharpness of the observation with a green light-microscope (a green filter or a yellow one superposed on the blue filter). The contrast depends on the way one carries out the differentiation.
The genus *Campylobacter* is badly colored by R4 Safranin, while the genus *Legionella* remains colorless.

The rinsing liquid for staining can be distilled, demineralized, or tap water.

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

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