FIX-RAL 555

REF. 362870

Fixation before differential staining of cellular structures

CE IVD

IFU024B

For professional use only.
Please read all information carefully before using this device.
IFU content may change, make sure you have the latest version available at my.ral-diagnostics.fr.

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Intended use

FIX-RAL 555 is intended to be used in combination with EOSIN-RAL 555 and BLUE-RAL 555 for the fixation and the differential staining of biological samples and cellular structures prior microscopic examination.

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

Principle

FIX-RAL 555 in combination with EOSIN-RAL 555 and BLUE-RAL 555 are fast-acting variation of May-Grünwald Giemsa staining.
In an aqueous buffered medium, this products association enables:

- A differential staining of blood smears (differential blood cell counting, morphological study of leukocytes, study of parasites) and medullary smears (myelograms)
- The detection of tissular and blood parasites in medical and in veterinary mycology
- The cytological and structural study of fixed and paraffin embedded tissue sections as well as fluids and punctions
- The cytological study of urines, spinal, and other fluids

The analysis of smears is identical to the one carried out with standard MGG staining.

Device description

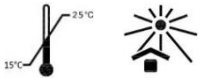
FIX-RAL 555
Clear purple solution

REF. 362870-1000	1 X 1 L
REF. 362870-2500	1 X 2.5 L

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

Storage and use conditions

Storage and use temperature: 15-25°C.
Storage and use conditions: away from light and heat sources.
Bottle shelf life before opening: refer to expiry date on the label.
Bottle shelf life after opening: refer to expiry date on the label and if the "period after opening" symbol is present take it into account.



Active components

FIX-RAL 555
Methanol - CAS 67-56-1: >80%

Hazard classification and safety information

FIX-RAL 555

Danger:
H225 - Highly flammable liquid and vapour.
H301+H311+H331 - Toxic if swallowed, in contact with skin or if inhaled.
H370 - Causes damage to organs.
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.
P264 - Wash hands thoroughly after handling.
P280 - Wear protective gloves, protective clothing, eye protection, face protection.
P301+P310 - IF SWALLOWED: Immediately call a POISON CENTER or doctor.
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 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, absolute ethanol, 90° ethanol and isopropanol and these following CellaVision RAL Diagnostics devices:
EOSIN-RAL 555 REF. 361640
BLUE-RAL 555 REF. 361650

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
The specimen must treat in accordance with procedures available in the laboratory and promulgated by national authorities.

Manual blood smear: Mix the tube by slow inversion and install a smearing droplet device. Invert the tube and lightly press the drop depositor onto a slide to deposit a small drop of blood (Fig. 1- slide A at step 1).
Using another slide tilted at 45° (Fig. 1- slide B at step 1), spread the blood by capillarity on the short edge (Fig. 1- steps 2 & 3) using a pushing motion (Fig. 1- step 4). A good quality smear does not reach the end of the slide and has a gradual decrease in thickness until the end is feathered. Allow the smear to air dry before fixing or staining.
NB: if you do not have a smearing droplet device, open the tube, and use a pipette to deposit a blood drop.

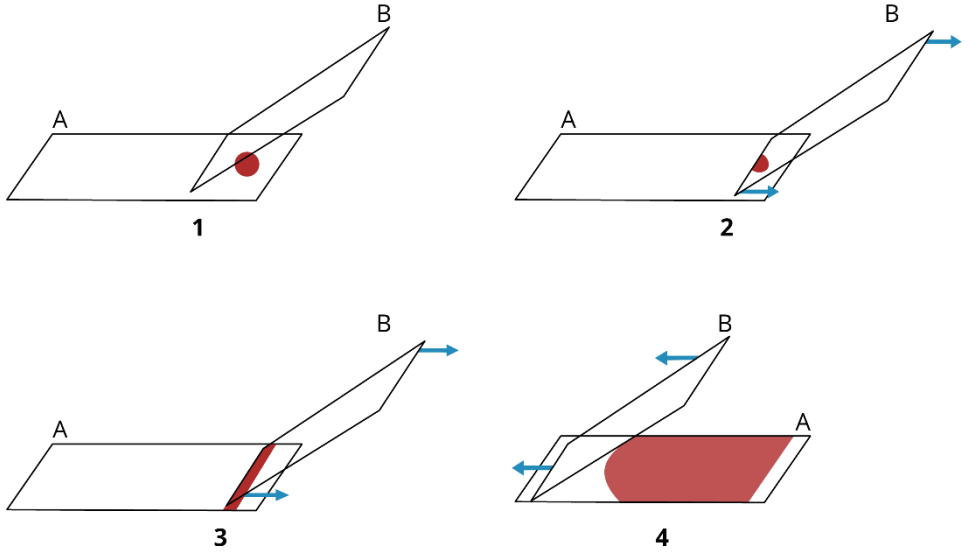


Figure 1. Schematic representation of performing a blood smear
A & B: slides, 1 – 4: steps 1 to 4

Manual bone marrow smear by crushing method: using a pipette deposit, a small amount of the sample on a microscope slide. Blot up blood excess to keep only shiny lumps. Cover the first slide with a slide. Squeeze and thin the sample by sliding and stretching to the end of the slide. A good quality smear does not reach the end of the slide. Discard the slide used for smearing. Allow the smear to air dry before fixing or staining.

Thick blood smear: take 2 µL of blood out of an EDTA tube and place them in the center of a slide. Spread the drop in a circle with the corner of another slide. Allow it to dry for 20 minutes in the open air or for 5 minutes in an incubator or for 2 minutes under a hair dryer. Cover the drop with very little tap water to hemolyze it. Once the hemoglobin has spread, get rid of the red water just inclining the slide. Rinse the drop very gently with tap water. The drop the gets a whitish appearance.

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.

Histological sections: dewax and et hydrate tissues sections in appropriate reagents before staining.

Spermocytograms: to avoid any detachment of the smear, make a smear that would be neither too fine, nor too thick and allow the smear to dry well: during several hours in air or on a hot plate.

Reagents and instruments preparation
No preparation needed. The solutions are ready to use. Transfer the solutions into staining baths as indicated in the protocols below.

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

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. Please refer to the title to know which case you are in. For the covering method, place slide on a stand with fixed smear on top. The processing time only considers the dipping time in the reagents.

Drain excess solution on filter paper at each solution change as shown on the schema (Fig.2).

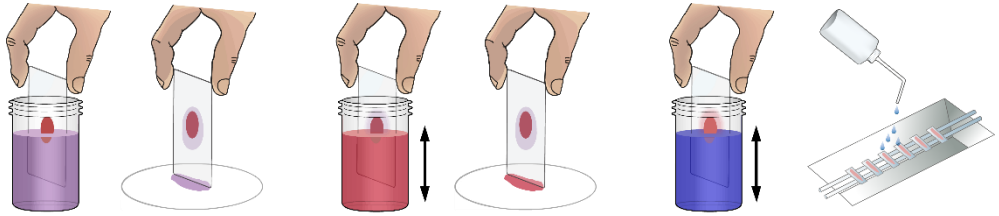


Figure 2. Schematic representation of performing Diff-Quik staining
1 – 3: steps 1 to 3

1. Dip slide in the FIX-RAL 555 according to the protocol and drain the excess solution on filter paper.
2. Dip slide in the EOSIN-RAL 555 according to the protocol and drain the excess solution on filter paper.
3. Dip slide in the BLUE-RAL 555 according to the protocol and rinse with distilled water.

Protocol for blood smear staining - Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 00: 15

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 05	Dip 5 X 1 second
Stain	EOSIN-RAL 555	00: 05	Dip 5 X 1 second
Stain	BLUE-RAL 555	00: 05	Dip 5 X 1 second
Rinse	Rinsing liquid	NA	Quickly
Dry	NA	≥03: 00	NA

Protocol for medullary smear staining - Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 03: 00

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	01: 00	NA
Stain	EOSIN-RAL 555	01: 00	
Stain	BLUE-RAL 555	01: 00	
Rinse	Rinsing liquid	NA	Quickly
Dry	NA	≥03: 00	NA

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

Processing time [hh: mm: ss]: 00: 01 :20

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	01: 00	NA
Stain	EOSIN-RAL 555	00: 10	Dip 10 X 1 second
Stain	BLUE-RAL 555	00: 10	Dip 10 X 1 second
Rinse	Rinsing liquid	NA	Quickly
Dry	NA	≥03: 00	NA

Protocol for histo-cytology samples staining - Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 00: 15

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 05	NA
Stain	EOSIN-RAL 555	00: 05	
Stain	BLUE-RAL 555	00: 05	
Rinse	Rinsing liquid	NA	Quickly
Dry	NA	≥03: 00	NA

Protocol for cyto-bacteriology of fluids, urines and cytopunctures - Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 00 :15

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 05	NA
Stain	EOSIN-RAL 555	00: 05	
Stain	BLUE-RAL 555	00: 05	
Rinse	Rinsing liquid	NA	Quickly
Dry	NA	≥03: 00	NA

Protocol for cyto-bacteriology of cerebrospinal fluid (CSF) samples staining - Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 01: 04

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	01: 00	NA
Stain	EOSIN-RAL 555	00: 02	Dip 2 X 1 second
Stain	BLUE-RAL 555	00: 02	Dip 2 X 1 second
Rinse	Rinsing liquid	NA	Quickly
Dry	NA	≥03: 00	NA

Protocol for fixed and paraffined tissues sections staining - Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 00 :23

Steps	Reagent	Time [mm: ss]	Indications
Stain	EOSIN-RAL 555	00: 05	NA
Stain	BLUE-RAL 555	00: 07	
Rinse	Rinsing liquid	NA	Briefly
Differentiate	90° ethanol	00: 01*	Shake in the bath until the slide gets the wished tint
Stop differentiation	Isopropanol	00: 10	NA
Mount	Mounting medium	≥03: 00	

** The differentiation step in ethanol 90° can be extended to 2 seconds.*
Dewax and et hydrate tissues sections in appropriate reagents before staining. Do not dip slides in the FIX-RAL 555 solution.

Protocol for cytology of punctions (breast and deep organs), effusion liquids of the serous membrane (pleura, peritoneum ...) staining - Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 00: 15

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 05	NA
Stain	EOSIN-RAL 555	00: 05	
Stain	BLUE-RAL 555	00: 05	
Rinse	Rinsing liquid	NA	Quickly
Dry	NA	≥03: 00	NA

Protocol for Plasmodium research in thick blood smear - Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 00: 07

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 01	NA
Stain	EOSIN-RAL 555	00: 03	Dip 3 X 1 second
Stain	BLUE-RAL 555	00: 03	Dip 3 X 1 second
Rinse	Rinsing liquid	NA	Very gently
Dry	NA	≥03: 00	NA

Protocol for blood film for Plasmodium research - Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 01: 04

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	01: 00	NA
Stain	EOSIN-RAL 555	00: 02	Dip 2 X 1 second
Stain	BLUE-RAL 555	00: 02	Dip 2 X 1 second
Rinse	Rinsing liquid	NA	Quickly
Dry	NA	≥03: 00	NA

Protocol for staining of tissular Protozoa (Leishmania, Toxoplasma, Microsporidiosis), Cryptosporidium, Pneumocystis carinii, fungi contributing to deep Mycosis - Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 02: 05

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	01: 00	NA
Stain	EOSIN-RAL 555	00: 25	
Stain	BLUE-RAL 555	00: 40	
Rinse	Rinsing liquid	NA	Quickly
Dry	NA	≥03:00	NA

Protocol for veterinary parasitology (Piroplasmosis, M.pachydermatis)- Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 00: 15

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 05	Dip 5 X 1 second
Stain	EOSIN-RAL 555	00: 05	Dip 5 X 1 second
Stain	BLUE-RAL 555	00: 05	Dip 5 X 1 second
Rinse	Rinsing liquid	NA	Quickly
Dry	NA	≥03: 00	NA

Protocol for Trichomonas research - Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 02: 15

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 15	NA
Stain	EOSIN-RAL 555	01: 00	
Stain	BLUE-RAL 555	01: 00	
Rinse	Rinsing liquid	NA	Quickly
Dry	NA	≥03:00	NA

Protocol for Microfilaria research - Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 00: 30

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 10	Dip 10 X 1 second
Stain	EOSIN-RAL 555	00: 10	Dip 10 X 1 second
Stain	BLUE-RAL 555	00: 10*	Dip 10 X 1 second
Rinse	Rinsing liquid	NA	Quickly
Dry	NA	≥03:00	NA

**The staining step in BLUE-RAL 555 can be extended to 20 seconds (20 x 1 second dips).*

Protocol for Helicobacter pylori research - Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 00: 22

Steps	Reagent	Time[mm: ss]	Indications
Stain	EOSIN-RAL 555	00: 07	NA
Stain	BLUE-RAL 555	00: 05	
Rinse	Rinsing liquid	NA	Dry on to filter paper
Differentiate	90° ethanol	00: 10	Dip and shake
Stop differentiation	Absolute ethanol	NA	NA
Dehydrate	Mounting medium	NA	2 baths

Dewax and et hydrate tissues sections in appropriate reagents before staining. Do not dip slides in the FIX-RAL solution.

Expected results

Blood or bone marrow smear

Nuclei / chromatin: +/- dense purple
Leukocytes cytoplasm without RNA: light pinkish
Granulocytes eosinophilic granules: orange- brown
Granulocytes basophilic granules: dark purple-blue
Granulocytes neutrophilic granules: +/- deep purple
Lymphocytes cytoplasm without RNA: pure blue
Lymphocytes cytoplasm without RNA: light blue
Lymphocytes azurophilic granules: red
Monocytes cytoplasm: grey-blue
Erythrocytes: light red
Platelets chromomere: purple-red
Platelets Hyalomere: bluish
Blood parasites nucleus (Plasmodium): red
Blood parasites cytoplasm (Plasmodium): blue

Spermocytograms

Head piece -Nucleus: purple
Head piece-Acrosome: pink
Midpiece: purplish pink
Flagellum: light pink

Assess in Percentage:
• abnormalities of the head, midpiece and flagellum
• agglutinates
• leucocytes, erythrocytes and cells

Parasitology and Mycology

Cytoplasms of host, fungic or parasitic eucaryot cells: blue to dark blue, depending on the ribosomal richness.
Nuclei: purple red

Helicobacter pylori on histological sections

Helicobacter pylori: dark blue
Nuclei: blue
Cytoplasm: pink to red
Collagen: very pale pink

Histo-cytology

Nuclei: red violet
Acidophilic cytoplasm: pink
Basophilic cytoplasm: blue
Collagen: pale pink
Erythrocytes: beige

If observed results vary from those expected, please contact CellaVision 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.
The following examples are for hematological and bacterial samples.

Hematological sample: CellaVision RAL Diagnostics recommends staining a freshly made blood smear with a normal WBC count and no known abnormal pathology at reagent renewal and for the first staining cycle 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).

Bacterial sample: CellaVision RAL Diagnostics recommend using a known bacteria sample for reagents quality control at reagents renewal, for each staining cycle or at least for the first staining cycle if a stain is performed multiple times daily.
The result is checked under a microscope, in comparison with the results obtained by the usual technique validated by the laboratory.
Staining results 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 CellaVision 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.

The FIX-RAL 555 contains methanol which is very hygroscopic, and must be therefore regularly refilled, namely in countries having high humidity). We advise you to put the lids back on the bottles at the end of staining cycles if you notice that FIX-RAL 555 evaporates quickly in your laboratory.

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

Products stability
Every CellaVision 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. If it is necessary to store the stained smears for several months or years, CellaVision RAL Diagnostics recommended mounting them with a coverslip, using a suitable mounting liquid and storing 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
	Batch code
	Serial number
	Catalogue reference
	Date of manufacture
	Use up to
	Unique device identifier
	Manufacturer
	Importer
	Entity distributing the medical advice in the region concerned
	CE marking device
	In vitro diagnostic medical device
	Authorised Representative in the European Community
	Authorised Representative in Switzerland
	Complies with UK guidelines
	Do not use if packaging is damaged
	Keep away from light Keep away form heat
	Temperature limit: 15-25°
	Temperature limit: 15-30°
	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
	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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Changes tracking

Date	Version	Changes
10/2023	IFU024B	Update in header and the following paragraphs: Storage and use conditions, Operating procedure and Expected results. Adjunction of legal representatives and GMED logo.
05/2022	IFU024A	IVDR (EU) 2017/746 compliance

Legal representatives

Countries	Address
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