Kit RAL Stainer 555

REF. 360250-0000

Fixation and differential staining of cellular structures

CE IVD

IFU056A-RAL

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

Table of contents

Intended Use

Kit RAL Stainer 555 is intended to be used in combination with the RAL Stainer instrument for the fixation and the differential staining of biological samples and cellular structures 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

The staining by Kit RAL Stainer 555 is a fast-acting variation of May-Grünwald Giemsa (MGG) staining. This product acts in buffered aqueous medium and allows a cytological study or microorganisms detection. The analysis of the staining is identical to that carried out with standard MGG staining.

Kit description

FIX-RAL 555
Clear purple solution
REF. 362870-0200 1 X 200 mL

EOSIN-RAL 555
Clear orange red solution
REF. 361640-0200 1 X 200 mL

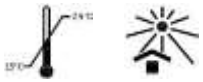
BUFFER-RAL 555
Clear colorless solution
REF. 361645-0200 1 X 200 mL

BLUE-RAL 555
Clear dark blue solution
REF. 361650-0200 1 X 200 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 opening: refer to the expiry date on the label.
Bottle shelf life after opening: 2 weeks or 300 slides.
Once opened, the duration of use overrules the expiry date



Active components

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

EOSIN-RAL 555
Eosin Y- CAS 17372-87-1: <0.1%
A mixture of 5-chloro-2-methyl-2H-isothiazol-3-one [EC No 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC No 220-239-6] (3:1) – CAS 55965-84-9: ≤0.005 %

BUFFER-RAL 555
Potassic mono phosphate - CAS 7778-77-0: ca 1%
Anhydrous disodic phosphate - CAS 7558-79-4: ca 2%
A mixture of 5-chloro-2-methyl-2H-isothiazol-3-one [EC No 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC No 220-239-6] (3:1) – CAS 55965-84-9: ≤0.005 %

BLUE-RAL 555
Methylene blue – CAS 61-73-4: < 0.1%

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 vapours. 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

EOSIN-RAL 555

Warning: H317-May cause an allergic skin reaction. H412 - Harmful to aquatic life with long lasting effects.
P261 - Avoid breathing dust/fume/gas/mist/vapours/spray. P280 - Wear protective clothing, protective gloves, eye protection. P333+P313 - If skin irritation or rash occurs: Get medical advice/attention. P362+P364 - Take off contaminated clothing and wash it before reuse.

CONT	5-chloro-2-methyl-2H-isothiazol-3-one/ 2-methyl-2H-isothiazol-3-one

BUFFER-RAL 555

Warning: H317 - May cause an allergic skin reaction. H412 - Harmful to aquatic life with long lasting effects.
P261 - Avoid breathing dust/fume/gas/mist/vapours/spray. P280 - Wear protective gloves, protective clothing, eye protection. P333+P313 - If skin irritation or rash occurs: Get medical advice/attention. P362+P364 - Take off contaminated clothing and wash it before reuse.

CONT	5-chloro-2-methyl-2H-isothiazol-3-one/ 2-methyl-2H-isothiazol-3-one

BLUE-RAL 555

No labelling applicable

The RFID label used is a passive short-range contactless memory chip (13,56MHz).

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, absolute ethanol, 90° ethanol, isopropanol and this following RAL Diagnostics device:
RAL Stainer REF. 405000

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 following examples are for hematological and bacterial sample preparations, 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.

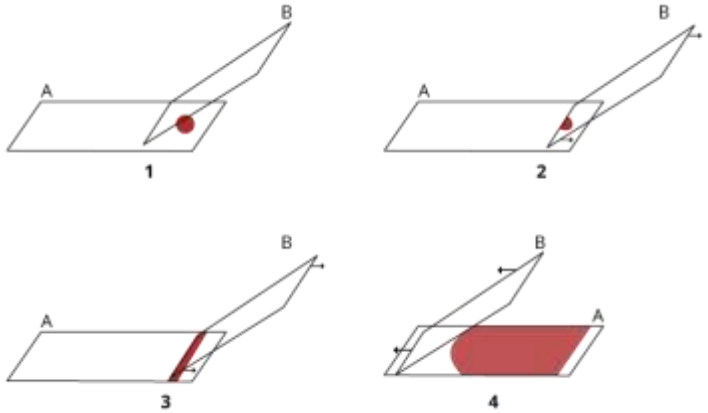


Figure 1. Schematic representation of performing a blood smear
A & B: slides, 1 – 4: steps 1 to 4

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.

Reagents and instruments preparation

No preparation needed. The solutions are ready to use and the reagents containers have been designed to be used for slides staining.
Place the 4 bottles labelled 1 to 4 in their respective set position and remove the caps and security rings.

The usual rinsing liquid for staining, validated by the laboratory, can be tap water, demineralized water, etc. Only de-ionized water is deprecated because it can cause a dysfunctionment of the RAL Stainer level sensor.

Protocols

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

An assortment of staining settings, recommended by RAL Diagnostics, are pre-set in the RFID tag.

Protocol for blood smear staining – Automated bath method- Manual microscopic analysis (initial settings)

Processing time: 32 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 07	With agitation
Stain	EOSIN-RAL 555	00: 07	
Rinse	BUFFER-RAL 555	00: 01	
Stain	BLUE-RAL 555	00: 07	
Rinse	Rinsing liquid	00: 10	
Dry	No	02: 00	No

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

Processing time: 3 min 11s

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	01: 00	Without agitation
Stain	EOSIN-RAL 555	01: 00	Without agitation
Rinse	BUFFER-RAL 555	00: 01	With agitation
Stain	BLUE-RAL 555	01: 00	Without agitation
Rinse	Rinsing liquid	00: 10	With agitation
Dry	No	02: 00	No

Protocol for Plasmodium research in thick drop - Automated bath method - Manual microscopic analysis

Processing time: 22 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 01	Without agitation
Stain	EOSIN-RAL 555	00: 05	With agitation
Rinse	BUFFER-RAL 555	00: 01	
Stain	BLUE-RAL 555	00: 05	
Rinse	Rinsing liquid	00: 10	
Dry	No	02: 00	No

Protocol for Plasmodium research in thin smear - Automated bath method - Manual microscopic analysis

Processing time: 1 min 19 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	01: 00	Without agitation
Stain	EOSIN-RAL 555	00: 04	With agitation
Rinse	BUFFER-RAL 555	00: 01	
Stain	BLUE-RAL 555	00: 04	
Rinse	Rinsing liquid	00: 10	
Dry	No	02: 00	No

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

Processing time: 2 min 16 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	01: 00	Without agitation
Stain	EOSIN-RAL 555	00: 25	
Rinse	BUFFER-RAL 555	00: 01	
Stain	BLUE-RAL 555	00: 40	
Rinse	Rinsing liquid	00: 10	With agitation
Dry	No	02: 00	No

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

Processing time: 32 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 07	With agitation
Stain	EOSIN-RAL 555	00: 07	
Rinse	BUFFER-RAL 555	00: 01	
Stain	BLUE-RAL 555	00: 07	
Rinse	Rinsing liquid	00: 10	
Dry	No	02: 00	No

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

Processing time: 2 min 26 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 15	Without agitation
Stain	EOSIN-RAL 555	01: 00	Without agitation
Rinse	BUFFER-RAL 555	00: 01	With agitation
Stain	BLUE-RAL 555	01: 00	Without agitation
Rinse	Rinsing liquid	00: 10	With agitation
Dry	No	02: 00	No

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

Processing time: 56 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 15	With agitation
Stain	EOSIN-RAL 555	00: 15	
Rinse	BUFFER-RAL 555	00: 01	
Stain	BLUE-RAL 555	00: 15	With agitation, can be extend to 30 seconds
Rinse	Rinsing liquid	00: 10	With agitation
Dry	No	02: 00	No

Protocol for cyto-bacteriology of urines fluids - Automated bath method - Manual microscopic analysis (initial settings)

Processing time: 32 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 07	With agitation
Stain	EOSIN-RAL 555	00: 07	
Rinse	BUFFER-RAL 555	00: 01	
Stain	BLUE-RAL 555	00: 07	
Rinse	Rinsing liquid	00: 10	
Dry	No	02: 00	No

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

Processing time: 1 min 19 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	01: 00	Without agitation
Stain	EOSIN-RAL 555	00: 04	With agitation
Rinse	BUFFER-RAL 555	00: 01	
Stain	BLUE-RAL 555	00: 04	
Rinse	Rinsing liquid	00: 10	
Dry	No	02: 00	No

Protocol for cytology of punctions (breast and deep organs), effusion liquids of the serous membrane - Automated bath method - Manual microscopic analysis (initial settings)

Processing time: 32 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 07	With agitation
Stain	EOSIN-RAL 555	00: 07	
Rinse	BUFFER-RAL 555	00: 01	
Stain	BLUE-RAL 555	00: 07	
Rinse	Rinsing liquid	00: 10	
Dry	No	02: 00	No

Protocol for fixed and paraffined tissues sections staining - Automated bath method - Manual microscopic analysis (initial settings)

Dewax and et hydrate tissues sections in appropriate reagents before staining on RAL Stainer. Do not dip slides in the FIX-RAL 555 solution.

Processing time: 24 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 00	Do not use
Stain	EOSIN-RAL 555	00: 05	Without agitation
Rinse	BUFFER-RAL 555	00: 01	With agitation
Stain	BLUE-RAL 555	00: 07	Without agitation
Rinse	Rinsing liquid	00: 10	With agitation
Dry	No	00: 00	No
Differentiate	90° ethanol	00:01	Can be extend to 2 seconds Shake in the reagent until gets the wished tint
Stop differentiation	Isopropanol	No	
Mount	Mounting medium	No	

Protocol for Helicobacter pylori research - Automated bath method - Manual microscopic analysis (initial settings)

Dewax and et hydrate tissues sections in appropriate reagents before staining on RAL Stainer. Do not dip slides in the FIX-RAL 555 solution.

Processing time: 33 s

Steps	Reagent	Time [mm: ss]	Indications
Fix	FIX-RAL 555	00: 00	Do not use
Stain	EOSIN-RAL 555	00: 07	With agitation
Rinse	BUFFER-RAL 555	00: 01	Without agitation
Stain	BLUE-RAL 555	00: 05	With agitation
Rinse	Rinsing liquid	00: 10	Without agitation
Dry	No	00: 00	No
Differentiate	90° ethanol	00:10	Dip and shake
Stop differentiation	Absolute ethanol	No	No
Dehydrate	Mounting medium	No	2 baths
Mount	Mounting medium	No	No

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 purplish-blue
Granulocytes neutrophilic granules: +/- intense 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: purplish -red
Platelets Hyalomere: bluish
Blood parasites nucleus: red
Blood parasites cytoplasm: blue

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 RAL Diagnostics technical service through your usual supplier for assistance.

Performance

The efficacy of the Kit RAL Stainer 555 reagents was evaluated in a hospital laboratory with 301 clinical samples (respiratory sample, urines, stools, biopsies, fungal cartography etc.).
The sensitivity of the Kit RAL Stainer 555 reagents was evaluated in comparison with the routine laboratory's reference technique: MGG in bath. All tests were performed in parallel under the same conditions.
The results obtained in this study show that the efficiency of the Kit RAL Stainer 555 is equivalent to the MGG routine method.

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.
The following examples are for hematological and bacterial samples.

Hematological sample: 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: 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 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.

According to the thickness of the smears or the specimens on slides, it may be necessary to adjust staining times.

The FIX-RAL 555 contains methanol which is very hygroscopic, and must be therefore regularly refilled, namely in countries having high humidity.
The color of EOSIN-RAL 555 solution may appear more or less dark, but the staining results are not affected either way.
The oxidation level of BLUE-RAL 555 is standardized during manufacture, but this level varies over time and in transferring small quantities of eosin from bottle to bottle.

The usual rinsing liquid for staining, validated by the laboratory, can be tap water, demineralized water, etc. Only de-ionized water is deprecated because it can cause a dysfunction of the RAL Stainer level sensor.
Decontamination of the rinsing container must be carried out according to the recommendations of the RAL Stainer operating manual.

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. If it is necessary to store the stained smears for several months or years, 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
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

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Change tracking

Date	Version	Changes
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RAL Diagnostics - Site Montesquieu - 33650 Martillac – France
T+33(0)5 57 96 04 04 - F +33 (0)5 57 96 04 55 - ral-diagnostics.fr / cellavision.com