Kit RAL StainBox BBM

REF. 360400-0000

Fixation and differential staining of cellular structures

CE IVD

IFU002D

For professional use only.
Read all the 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

Kit RAL StainBox BBM is intended to be used in combination with RAL StainBox instrument for the fixation and the differential staining of 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

The BBM panoptic staining allows to perform blood cell and bone marrow counting, it is realized by using successively five reagents: R1, R2, R3, R4 and R5.
R1, formulated with ethylic alcohol, is a mixture of neutral stains. It allows the smear fixation and prepares the staining, especially of hydrosoluble elements such as basophilic granules.
Those stains are inactive in alcoholic medium, and only react selectively when released in R2 and R3 solutions. This releasing generates the precipitation of neutral stains, leading to the staining of erythrocytes, cytoplasm of neutrophilic granulocytes as well as eosinophilic granules. R4 is a blue aqueous solution which stains the cytoplasm of monocytes and lymphocytes. R4 also eases the metachromasia process as it colors azurophilic granules red. Eventually, R5 removes excess of stain and participates to the differentiation of cellular elements thanks to the action of specially selected rinsing agents.
The successive action of R1, R2, R3, R4 and R5 brings the violet color (typical Romanowsky-Giemsa effect), particularly visible in chromatin, platelets, and neutrophilic granules.

Kit description

R1
Clear dark blue solution
REF. 313595-0250 1 X 230 mL

R2
Clear colorless solution
REF. 3135752A0250 1 X 230 mL

R3
Clear colorless solution
REF. 3135753A0250 1 X 230 mL

R4
Clear dark blue solution
REF. 313565-0250 1 X 230 mL

R5
Clear colorless solution
REF. 313605-0250 4 X 230 mL

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 the expiry date on the label.
Bottle shelf life after opening: 4 weeks after opening or 300 slides, refer to the expiry date on the label and if the "period after opening" symbol is present take it into account.

Active components

R1
May-Grünwald: 0.1%
Methylene azure I blue – CAS - 531-55-5: 0.05%

R2 and R3
Potassic mono phosphate - CAS 7778-77-0: < 1 %
Anhydrous disodic phosphate - CAS 7558-79-4: < 1 %

R4
Methylene blue – CAS - 61-73-4: < 0.25%

R5
Potassic mono phosphate - CAS 7778-77-0: 0.03%
Anhydrous disodic phosphate - CAS 7558-79-4: 0.03%

Hazard classification and safety information

R1

Danger:
H225-Highly flammable liquid and vapour.
P210-Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

R2 and R3

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

R4

No labelling applicable

R5

Warning:
H226 - Flammable liquid and vapour.
H317 - May cause an allergic skin reaction.
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.
P261 - Avoid breathing dust / fume / gas / mist / vapours / spray.
P280 - Wear protective gloves, protective clothing, eye protection.
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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

The RFID tag 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 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 and RAL StainBox REF. 402000

This equipment may vary depending on the protocol. 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 be treated in accordance with procedures available in the laboratory and required 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.

***Note**:if you do not have a smearing droplet device, open the tube, and use a pipette to deposit a blood drop.*

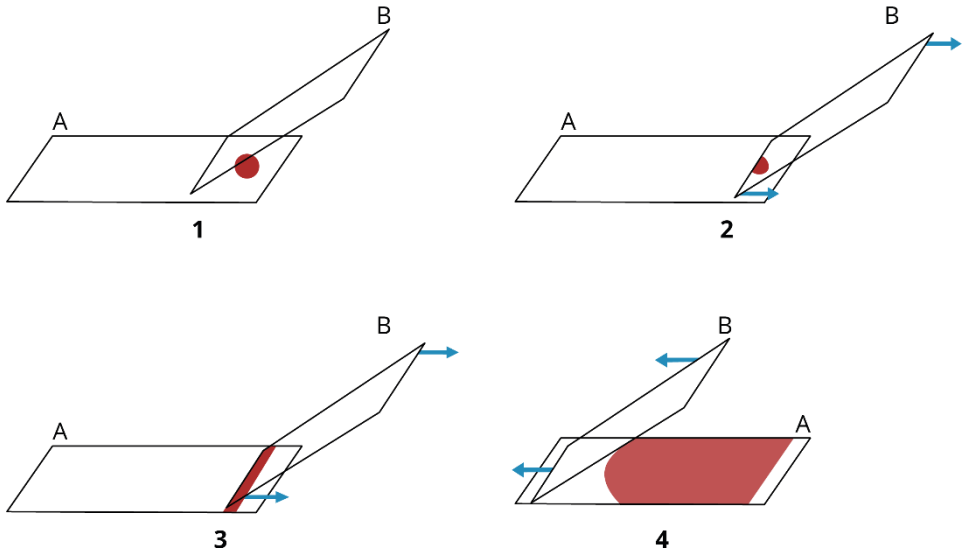

Figure 1. Schematic representation of performing a blood smear.
A & B: slides, 1 – 4: steps 1 to 4

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.

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.

On the StainBox main screen, press the button  to open all the StainBox lids. Remove the bottle caps and security rings 1 to 5 and screw them on their respective supports. Then be sure to place the bottles in their location (Table 1. Bottles support and location). Close all the lids manually and follow the instructions on the screen.

Station	1	2	3	4	5
Bottle	R1	R2	R3	R4	R5
Support					
LED colour	Flickering LED	Pink LED	Blue LED	Yellow LED	White LED

Table 1. Bottles support and location.

Figure 2. Schematic representation of staining steps of the RAL StainBox instrument.

1- Place the slide-holder in the first station.
2- At the end of the countdown, lids 1 & 2 will open. Transfer the slide-holder to station 2. Do not agitate.
3- Close lids 1 & 2.
4- At the end of the countdown, lids 2 & 3 open. Agitate the slides in station 2 (according to the protocol in use).
5- Transfer the slide-holder over to station 3. Agitate the slides in station 3 (according to the protocol in use).
6- Close lids 2 & 3.
7- At the end of the countdown, lids 3 & 4 open. Agitate the slides in station 3 (according to the protocol in use).
8- Transfer the slide-holder over to station 4. Agitate the slides in station 4 (according to the protocol in use) and close lids 3 & 4. At the end of the countdown, lids 4 & 5 open.
9- Transfer the slide-holder to station 5, close the lid 4.
10- Let the lid 5 open and agitate according to the protocol in use.
11- At the end of the countdown transfer the slide-holder to station 6 and close the lids 5 & 6.
12- When finished, remove the stained slides from station 6 and close the lid. The slides are ready to analyze.

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. Refer to the title to know which case you are in. The processing time only considers the contact time with the reagents.

Staining settings, recommended by CellaVision RAL Diagnostics, are pre-set in the RFID tag.

Protocol for blood samples - Manual bath staining method - CellaVision® DC-1 automate analysis

Processing time [hh: mm: ss]: 00: 11: 30

Steps	Reagent	Time [mm: ss]	Indications
Fix and pre-stain	R1	06:00	Without agitation
Stain	R2	01:00	Agitate in the bath, 5 to 10 times at the end of countdown*
Stain	R3	02:00	Agitate in the bath 5 to 10 times at the beginning and the end countdown*
Stain	R4	00:30	
Rinse	R5	02:00	
Dry	N/A	03:00	N/A

** Start agitation at lid opening.*

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

Processing time [hh: mm: ss]: 00: 09: 45

Steps	Reagent	Time [mm: ss]	Indications
Fix and pre-stain	R1	06:00	Without agitation
Stain	R2	01:00	Agitate in the bath at the end of countdown
Stain	R3	02:00	
Stain	R4	00:30	
Rinse	R5	00:15	Agitate continuously in the bath during countdown
Dry	N/A	03: 00	N/A

Protocol for bone marrow samples - Manual bath staining method - Manual microscopic analysis

Processing time [hh: mm: ss]: 00: 19: 45

Steps	Reagent	Time [mm: ss]	Indications
Fix and pre-stain	R1	15:00	Without agitation
Stain	R2	03:00	Agitate in the bath, 3 to 5 times at the end of countdown
Stain	R3	N/A	Do not dip in the bottle
Stain	R4	01:30	Agitate in the bath, 3 to 5 times at the end of countdown
Rinse	R5	00:15	Agitate continuously in the bath during countdown
Dry	N/A	03: 00	N/A

***Note**: In case of refringence/water artefact phenomena, pre-fix the slides 2min in a bath of absolute ethanol before staining. Directly start the staining after pre-fixation step without drying the slides.*

Expected results

Nuclei / chromatin: purple
Granulocytes cytoplasm: light purplish-pink
Granulocytes eosinophilic granules: orangey
Granulocytes basophilic granules: dark blue
Granulocytes neutrophilic granules: +/- deep purple
Lymphocytes cytoplasm with RNA: pure blue
Lymphocytes cytoplasm without RNA: light blue
Lymphocytes azurophilic granules: red
Monocytes cytoplasm: cloudy blue
Erythrocytes: reddish-pinkish
Platelets chromomere: purplish-red
Platelets hyalomere: bluish
Blood parasites nucleus (Plasmodium): red
Blood parasites cytoplasm (Plasmodium): blue

If observed results vary from those expected, contact CellaVision RAL Diagnostics technical service through your usual supplier for assistance.

Performance

The performance of the Kit RAL StainBox BBM reagents was evaluated in a hospital laboratory with 442 slides (blood and bone marrow).
The sensitivity of the Kit RAL StainBox BBM 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 StainBox BBM is satisfying to the MGG routine method.
The Kit RAL StainBox BBM reagents enable cell structure staining and microscopic analysis.
As they do not allow the detection of analytes, analytical performance is not applicable to these reagents.

These medical devices are based on scientific validity (scientific peer-reviewed literature) and demonstration of clinical performance through experience gained from routine diagnostic testing, and the regular evaluation of these performances under Post Market Performance Follow-up (PMPF), to ensure that they continue to meet the expected performance and safety standards.

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.

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 each day. Slides stained for quality control purposes should be checked to ensure that they are satisfactory for the intended test (properly stained and free of precipitates).
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.

Procedure notes
To prevent products degradation, comply with the storage and handling recommendations specified in this manual.

Kit RAL StainBox BBM allows to process up 300 slides.

R2 and R3: The use of the RAL StainBox MCDh kit generates 2 phases in the bottle. To ensure optimum staining quality throughout the shelf life of the kit agitate the stoppered bottle by turning it over to homogenize the product at the beginning and the end of each working day or if the reagent has not been used for a period longer than 8 hours.

R5: It is strongly recommended to change the bottle 5 (R5) every week or every 75 slides. At each kit replacement, be sure to remove inviolability rings and caps before screwing the staining devices.

In case of refringence/water artefact phenomena, pre-fix the slides 2min in a bath of absolute ethanol before staining. Directly start the staining after pre-fixation step without drying the slides.

The first staining cycle may appear slightly lighter than the final color shade. If necessary, you can prime staining with bare slides or with a smear without analyze them.

Product 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 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
	Authorised Representative in United Kingdom
	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
03/2025	IFU002D	Update in the following paragraph: Performance and Recommendations, notes, and troubleshooting. Removal of the GMED logo.
07/2024	IFU002C	Update in the following paragraphs: Operating procedure, User quality control and Table of symbols and abbreviations. Add of CH-REP and UK-REP symbols.
05/2023	IFU002B	Update in the header and the following paragraphs: Storage and use conditions, Active components, Operating procedure, Expected results, Recommendations, notes and troubleshooting. Add of legal representatives and GMED logo
05/2022	IFU002A	Update according to IVDR (EU) 2017/746

Legal representatives

Countries	Address
UK REP	Qavis UK Ltd, company N° SC679796, 56-66 Frederick Street Edinburgh, EH21LS, United Kingdom
CH REP	MedEnvoy Switzerland, Gotthardstrasse 28, 6302 Zug Switzerland