Kit RAL StainBox BBM

REF. 360400-0000

Fixation and differential staining of cellular structures

CE IVD

IFU002A-RAL

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

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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, 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 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

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

R 2
Clear colorless solution
REF. 3135752A0250 1 X 230 mL

R 3
Clear colorless solution
REF. 3135753A0250 1 X 230 mL

R 4
Clear dark blue solution
REF. 313565-0250 1 X 230 mL

R 5
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

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: 4 weeks after opening or 300 slides
Once opened, the duration of use overrules the expiry date

Active components

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

R2 and R3
Potassic mono phosphate - CAS 7778-77-0: ca 0.05%
Anhydrous disodic phosphate - CAS 7558-79-4: ca 0.09%

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

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

Hazard classification and safety information

R 1

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.
P280 - Wear protective clothing, protective gloves, eye protection. P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.

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

R 4

No labelling applicable

R 5

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. 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

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 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 and RAL StainBox REF. 402000,

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

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.

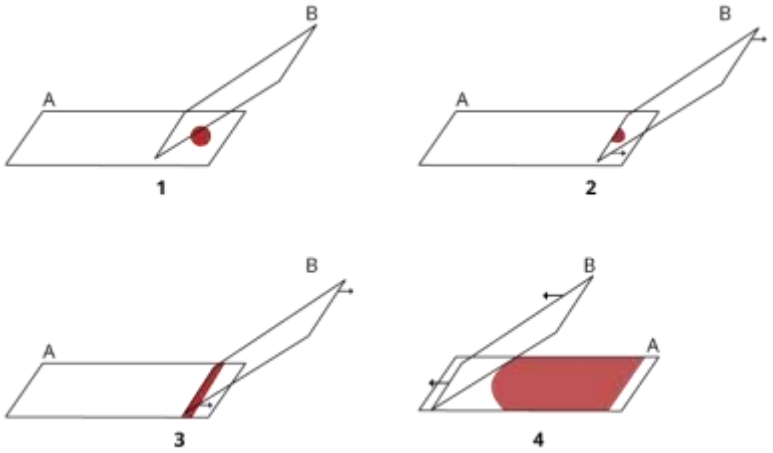

Figure 1. Schematic representation of performing a blood smear
A & B: slides, 1 – 4: steps 1 to 4

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.

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 in the 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 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 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 in 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.
13- .

Protocols

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

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

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

Processing time: 11 min 30

Steps	Reagent	Time [mm: ss]	Indications
Fix and pre-stain	R1	06:00	No
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	No	03:00	No

* Start agitation at lid opening.

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

Processing time: 09 min 45

Steps	Reagent	Time [mm: ss]	Indications
Fix and pre-stain	R1	06:00	No
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	No	03: 00	No

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

Processing time: 19 min 45

Steps	Reagent	Time [mm: ss]	Indications
Fix and pre-stain	R1	15:00	No
Stain	R2	03:00	Agitate in the bath, 3 to 5 times at the end of countdown
Stain	R3	No	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	No	03: 00	No

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: +/- dense 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: pinkish-beige
Platelets chromomere: purplish-red
Platelets hyalomere: bluish
Blood parasites nucleus: red
Blood parasites cytoplasm: blue

If observed results vary from those expected, please contact 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 300 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.

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 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).

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 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.

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
Σ	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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Changes tracking

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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