SUDAN BLACK B

Cytochemical Staining in Blood or Bone Marrow Smears for differentiating granulocytic leukemias from monocytic leukemias

10 x 4 tests **REF 3099**

PREFACE

The kit has to the been designed to reduce the reagents volume and minimize the exposure of the operator chemicals, to simplify the procedure and the disposal of the reagents. Based on current knowledge, the least toxic and polluting reagents were used in the kit.

PRINCIPLE

The reaction detects several intracellular lipids, including neutral fats, phospholipids and sterols, as fats in general can dissolve some stains which are removed from their alcoholic solution.
After staining, black color lipidic granules appear in all granulocytes with progressive increase in positive reaction from myeloblast to mature granulocyte. Lipidic granules are also present in Auer's bodies and may sometimes appear as a fine cytoplasmatic dispersion in monocytes. Lymphocytes, lymphoblasts and erythroblasts are negative to Sudan Black B. The presence of granules is evaluated under the microscope.
The kit is used to identify the origin of monocytic cells and thus to distinguish between granulocytic and monocytic leukemia.

REAGENTS AND MATERIALS

Kit components:	REF 3099
***REAGENT 1** Sudan black B	**1 x 30 mL**
***REAGENT 2** Buffer	**1 x 20 mL**

TOXICITY: the buffer contains phenol, a corrosive substance which causes burns. In case of contact with the eyes wash immediately with plenty of water and consult a doctor.

PLATES Disposable multi well (4 wells in each plate)	**10**
COVER in black color for the plates	**1**

(*) Dangerous reagents are market by an asterisk. Refer to MSDS.

STABILITY: protected from light, sealed and stored at room temperature, reagents are stable up to the expiration date on the label.

REAGENTS REQUIRED NOT SUPPLIED

FIXATIVE:		
Preparation:	formaldehyde 37%	1 volume
	absolute ethanol	9 volumes
COUNTERSTAINING:	Giemsa solution	

MATERIALS REQUIRED NOT SUPPLIED

400x or 1000x **microscope** for slide reading.
Pipettes with disposable tips or graduated Pasteur pipettes for sampling and dispensing the reagents.
Tube with cap
Timer
Thermostat
Deionized water

SAMPLE

Blood (preferably from capillary) or bone marrow smears.
Blood samples may be collected in EDTA or heparin. Samples can be stored at room temperature (18-26°C) and protected from dust for several days, without significant variation in activity.
Fixed slides can be stored for many weeks.

MANUAL ASSAY PROCEDURE

A) FIXATION OF THE SLIDES (see notes)
1. Fix the air-dried slides for 1 minute in the fixing solution.
2. Wash both sides of the slide in plenty of de-ionized water, drain it and wait till it is dry.

B) PREPARATION OF THE WORKING SOLUTION

Let the reagents reach room temperature before use.
1. Transfer 3 mL of Reagent 1 into a tube.
2. Add 2 mL of Reagent 2 into the tube.

Put the cap and mix well.
STABILITY: the working solution must be used right after it is prepared.

C) SUDAN BLACK B REACTION

1. Place the needed multi-well plates on a flat surface. Each plate and each bottle of working solution allow to run 4 determinations.
2. Put the slides on the plate with the smear facing downwards, towards the bottom of the well, to ensure that the working solution come into contact with the smear.
3. Put the slide against one of the two long edges of the well. Between the longer side of the slide and the well, there will be a long groove into which the working solution will be injected.
4. Take 1 mL of working solution using a pipette or Pasteur pipette. Insert the tip into the central area of the groove and slowly inject the working solution. The solution will spread in the well, coming into contact with the smear. Less than 1 mL is enough to fill the well. Proceed the same way with the other slides.
5. Place the plate in a thermostat at 37°C and cover it with the black cover to protect it from light. If more plates are used, put them one over the other before covering. Incubate for 15 minutes. Alternatively, incubate at room temperature (18-26°C) for 30 minutes.
6. Remove the slides with tweezers or fingers (wearing disposable gloves) and rinse them in running water. To facilitate this step, gently press one end of the slide so that the other one lifts up.
7. Washed and dried plates can be used for slide storage.

D) COUNTERSTAINING (see notes)
1. Counterstain in Giemsa solution for 10 minutes.
2. Rinse with running water, dry and read under the microscope.

RESULTS

The appearance of black granules inside the cell indicates the presence of fats.

PATHOLOGY

The reaction is positive in mature and immature myeloid cells, while sudanophilia is hard to be observed in lymphoid cells.
The method is therefore useful for differential diagnosis and classification of acute leukemia.

NOTES

Plates can be used for fixing and counterstaining the smear. In this case, put the slides as described in section C) and inject the fixing solution or the dye into the groove instead of the working solution. Proceed as described in sections A) and D) for fixation, counterstaining, and washes.

WASTE DISPOSAL

Dispose of reagents and used materials according to the regulations of your country.

BIBLIOGRAPHY

Available upon request

MANUFACTURER

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalogue number
	temperature limits
	use by
	caution
	read instructions for use

IVD CE

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