α-NAPHTHYL ACETATE ESTERASE LEUKOCYTE

Cytochemical staining on blood or bone marrow smears for the differential diagnosis of leukemias

10 x 4 test **REF 3089**

PREFACE

The kit has been designed to reduce the reagents volume and minimize the exposure of the operator to the 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

Blood or bone marrow smears are incubated with α-naphthyl acetate and pararosaniline. In the presence of α-naphthyl acetate esterase, a red precipitate forms in the cytoplasm of cells.
This reaction is mildly positive in monocytes, reticular cells, and megakaryocytes, weakly positive in lymphocytes, and even less positive in plasma-cell elements.
The reaction is negative in granulocytic cells.
The presence of the colored precipitate is evaluated under an optical microscope.
The kit is used to identify cells of monocytic origin and thus differentiate granulocytic from monocytic leukemias.

REAGENTS AND MATERIALS

Kit components	REF 3089
* **REAGENT 1** Sodium nitrite (lyophilized) TOXICITY: Toxic substance if ingested.	**10 vials**
* **REAGENT 2** Pararosaniline 1,5 g/L TOXICITY: Toxic substance if in contact with skin and ingestion. Keep away from light.	**1 x 10 mL**
* **REAGENT 3** Buffer 60 mmol/L	**1 x 45 mL**
* **REAGENT 4** α-naphtyl acetate TOXICITY: Toxic substance if inhaled	**1 x 2 mL**
MULTI-WELL PLATES (4 wells for each plate)	**10**
Black **COVER** for the plates	**1**

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

STABILITY: sealed and stored at 2-8°C reagents are stable up to the expiration date on the label.

REAGENTS REQUIRED BUT NOT PROVIDED

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

REQUIRED BUT NOT PROVIDED

Optical microscope 400x or 1000x for slide reading.
Pipettes with disposable tips or Pasteur pipettes for sampling and dispensing the reagents.
Thermostat set at 37°C, reduces incubation times.
Timer.
Deionized water.

SAMPLE

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

PROCEDURE

A) FIXATION OF SLIDES (see notes)

1. Fix the air-dried slide in the fixing solution for 1 minute.
2. Wash both sides of the slide in plenty of deionized water, drain it, and wait for it to dry.

The recommended fixative contains formaldehyde. Even a small amount of formaldehyde on the slides can inhibit the enzyme. Therefore, it is necessary to completely remove the fixative.

B) PREPARATION OF THE WORKING SOLUTION

Let the reagents reach room temperature before use. Unscrew the screw cap and gently remove the rubber cap from a bottle of Reagent 1.

1. Add 1 mL of Reagent 2 to the vial of Reagent 1. Replace the rubber cap and shake by inversion until the lyophilized reagent is completely dissolved. Wait for 2 minutes.
2. Reopen the vial of Reagent 1 and add 4 mL of Reagent 3.
3. Finally, add 0.1 mL of Reagent 4. Replace the rubber cap and shake by inversion.

STABILITY: The working solution should be used immediately after preparation.

C) REACTION OF α-NAPHTHYL ACETATE ESTERASEI

1. Put the needed multi-well plates on a flat surface. Each plate and each bottle of working solution allow to run 4 determinations.
2. Place the slides on the plate with the smear facing down, 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. Pipette or use a Pasteur pipette to take 1 mL of working solution. Insert the tip of the pipette or Pasteur pipette 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 37°C thermostat 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 them with the lid. Incubate for 15 minutes. Alternatively, if a thermostat is not available, incubate for 20 minutes at room temperature (18-26°C).
   Using tweezers or fingers (wearing disposable gloves), remove the slides and rinse them in running tap water. To facilitate this step, gently press one end of the slide so that the other one lifts. Washed and dried plates can be used for slide storage.

D) COUNTERSTAINING (see notes)

1. Counterstain with Giemsa solution for 10 minutes.
2. Rinse in running tap water, dry, and examine under the microscope. Experience in cytochemical techniques allows for the evaluation of slides without counterstaining.

RESULTS

The enzymatic activity is revealed by the presence of red granules in the cell cytoplasm.

PATHOLOGY

Used for identifying monocytes and for differentiating various types of acute leukemia. It shows a positive result in reticulosis and some non-Hodgkin lymphomas.

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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