α-NAPHTHYL BUTYRATE ESTERASE LEUKOCYTE

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

10 x 4 tests **REF 3091**

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 butyrate and pararosaniline. The development of a reddish-brown pigmented insoluble compound in the cytoplasm indicates the presence of butyrate esterase in monocytic-macrophagic cells. The presence of the stained compound in the cells is evaluated under the microscope. The kit is used for classification of leukemic forms. In some cases of myelodysplasia and myelomonocytic leukemia, a positive enzymatic reaction may be found in some myeloid cells.

REAGENTS AND MATERIALS

Kit components:	REF 3091
***REAGENT 1** Sodium nitrite (lyophilized) TOXICITY: toxic substance. Do not swallow	**10 vials**
***REAGENT 2** Pararosaniline ≥ 2,0 g/L TOXICITY: toxic substance if in contact with skin and ingestion. Keep away from light.	**1 x 10 mL**
REAGENT 3 Buffer 74 mmol/L	**1 x 45 mL**
***REAGENT 4** α-Naphthyl butyrate (suspension)	**1 x 5 mL**
PLATES Disposable multi well plates (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: 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: Methylene Blue or Giemsa solution.

REQUIRED BUT NOT PROVIDED

400x or 1000x **microscope** for slide reading
Pipettes with disposable tips or Pasteur pipettes for sampling and dispensing the reagents.
Timer
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) for several days, protected from dust, without any significant variation in activity.
Fixed slides can be stored for many weeks.

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 and wait till it is dry. The recommended fixative contains formaldehyde. Even a small amount of aldehyde on the slides may 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 carefully remove the rubber cap from the vial of Reagent 1.

1. Draw 1 mL of Reagent 2 and add it to the vial of Reagent 1. Replace the cap and gently shake by inversion until the lyophilized reagent is dissolved. Wait for 2 minutes.
2. Reopen the vial of Reagent 1 and add 4 mL of Reagent 3.
3. Reagent 4 contains a suspension: **shake the vial well** before taking 0.5 mL and adding it to Reagent 1. Replace the cap and shake well by inversion.

STABILITY: the working solution must be used right after it is prepared.

C) α-NAPHTHYL BUTYRATE ESTERASE REACTION

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 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. Cover the plate with the lid to protect it from light. If more plates are used, put them one over the other before covering. Incubate for 15 minutes at room temperature (18-26°C).
6. Remove the slides with tweezers or fingers (wearing disposable gloves) and rinse them in running tap water. To facilitate this step, gently press one end of the slide so that the other one lifts up. Washed and dried plates can be used for slide storage.

D) COUNTERSTAINING (see notes)

1. Counterstain in Methylene blue solution or in Giemsa solution for 10 minutes.
2. Rinse in running tap water, dry and read under the microscope.

RESULTS

The development of a reddish-brown pigmentation reveals the enzymatic activity.
The pigmentation of the enzymatic reaction which indicates the presence of α-Naphthyl butyrate esterase, is more evident if methylene blue solution is used to counterstain instead of Giemsa solution.
Giemsa solution allows a better definition of the cells, but pigmentation of the enzymatic reaction may be less evident, because it may be attenuated by the staining of the cells by the Giemsa solution.

PATHOLOGY

This reaction is used to classify monocytic-macrophagic cells, which evidence a diffused reddish-brown coloration. The reaction is useful to distinguish acute myelomonocytic leukemia (where monocytes and blasto have a positive esterase reaction) from acute granulocytic leukemia (where blasto and promyelocites are negative to the reaction). In some cases of myelodysplasia and myelomonocytic leukemia, a positive enzymatic reaction may be found in some myeloid cells.

NOTES

Disposable 4 well plates can also be used to fix and counterstain. In this case, put the slides as described in paragraph C) and inject the fixative or the stain instead of the working solution. Proceed as described in paragraphs A) and D) for fixing, counterstaining and washes.

WASTE DISPOSAL

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

BIBLIOGRAPHY

Available upon request.

MANUFACTURER

FAR
Via Fermi, 12 - 37026 Pescantina - VERONA - ITALY
phone +39 045 6700870 website http://www.fardiag.com
e-mail: order@fardiag.com e-mail: fardiag@fardiag.com

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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