NAPHTOL AS-D CHLOROACETATE ESTERASE LEUKOCYTE

Cytochemical staining on blood or bone marrow smears for the differentiation of granulocytic leukemia from monocytic leukemia

10 x 4 tests **REF 3092**

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 naphthol AS-D chloroacetate and pararosaniline. A red color precipitate in cell cytoplasm reveals the presence of naphthol AS-D chloroacetate esterase. This reaction is positive only in granulocytic cells, from myeloblasts to mature granulocyte. The reaction is weak or absent in monocytes and lymphocytes. The presence of the stained precipitate is evaluated under the microscope. The kit is used to identify cells of granulocytic origin and consequently to distinguish between granulocytic and monocytic leukemia.

REAGENTS AND MATERIALS

Kit components:	REF 3092
***REAGENT 1** Sodium nitrite (lyophilized)	**10 vials**
TOXICITY: Toxic substance. Do not swallow.	
***REAGENT 2** Pararosaniline	**1 x 10 ml**
***REAGENT 3** Buffer	**1 x 45 ml**
***REAGENT 4** Naphthol AS-D chloroacetate	**1 x 2 ml**
TOXICITY: toxic substance if inhaled	
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: 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.	

MATERIALS REQUIRED BUT NOT PROVIDED

400x or 1000x **microscope** for slide reading.
Pipettes with disposable tips or graduated Pasteur pipettes for sampling and dispensing the reagents.
Thermostat set at 37°C, reduces test incubation times.
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), protected from dust, for several days without any 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. The recommended fixative contains formaldehyde. Even a small quantity 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 a vial 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 and add 4 mL of Reagent 3. Close the vial and shake well.
3. Reopen the vial and add 0.1 mL of Reagent 4. Close the vial and shake well.

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

C) AS-D CHLOROACETATE ESTERASE 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, if a thermostat is not available, incubate for 20 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 Giemsa solution for 10 minutes.
2. Rinse in running tap water, dry and read under the microscope. Experience in cytochemical techniques allows for the evaluation of slides without counterstaining.

RESULTS

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

PATHOLOGY

This reaction helps to classify acute and chronic leukemia of myeloid type.

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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