PERODYDASE LEUKOCYTE

Cytochemical staining on blood or bone marrow smears for identification of myelocytic and monocytic cells

10 x 4 tests **REF 3090**

PREFACE

The kit has been designed to reduce the reagents volume, 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

In presence of endocellular peroxydase, diaminebenzidine (DAB) is oxidized by hydrogen peroxide and forms a brown color precipitate following the reaction:
DAB + H_2O_2 → oxidized DAB (brown color)
The presence of the precipitate in the cells is evaluated under the microscope.
The kit is used to evaluate if immature cells are granulocyte or monocyte. It shows a positive result with myelocyte and monocyte cells.

REAGENTS AND MATERIALS

Kit components:	REF 3090
(*) **REAGENT 1** Diamine-benzidine (freeze-dried)	**10 vials**
REAGENT 2 Hydrogen peroxide	**1 x 10 mL**
PLATES Disposable multi well plates (4 wells in 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

400x or 1000x **microscope** for slide reading.
Pipette with disposable tip or graduated Pasteur pipette for sampling and dispensing the reagents.
25 mL tube with cap.
Deionized water
Distilled 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 some days, protected from dust, 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 slide 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.
1. Transfer 0.1 mL of Reagent 2 (hydrogen peroxide 3%) to the 25 mL tube.
2. Transfer 25 mL of distilled water to the 25 mL tube. Close the cap of the tube and gently shake.
This is the working solution: a 0.012% hydrogen peroxide solution.
Keep the vial of Reagent 2 tightly closed.
3. Unscrew the screw cap and gently remove the rubber cap from a vial of Reagent 1.
4. Add 4 mL of 0.012% diluted hydrogen peroxide to the vial of Reagent 1.
5. Replace the rubber cap and gently shake by inversion until the lyophilic reagent is completely dissolved.

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

C) PEROXIDASE 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 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. Take 1 mL of working solution using a 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 black cover 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.
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 in running tap water, dry and read under the microscope.

The evaluation of the slides may be performed without counterstaining by experienced personnel.

RESULTS

A diffused brown color with variable intensity and with presence of brown granules proves the enzymatic activity.

PATHOLOGY

The following cells are positive to peroxydase reaction:
1. granulocytes with granulations which are sometimes bright with intense and progressive positivity, from myeloblast to mature granulocyte
2. monocytes may show thin granulations.
Peroxidase reaction is negative in other hematopoietic lineages.

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

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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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