

# ALKALINE PHOSPHATASE LEUKOCYTE

Cytochemical staining on blood or bone marrow smears for the classification of lymphocytic leukemias

10 x 4 tests

REF 3094

## 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

Peripheral blood or bone marrow smears are incubated at room temperature in a solution containing Naphthol AS-MX phosphate and Fast Blue BB salt. In the presence of phosphatase, a bluish color precipitate forms in granulocyte cytoplasm. Intensity and pattern of stained granules in cells are evaluated under the microscope. The kit is designed to classify lymphocyte leukemia. High Leukocyte Alkaline Phosphatase (PAL) is found in reactive granulocytosis, in pregnancy and in real polycythemia. Low PAL is found in chronic myeloid leukemia.

## REAGENTS AND MATERIALS

Kit components:

**REAGENT 1** Fast Blue BB salt (lyophilized)

REF 3094

10 vials

**REAGENT 2** Phosphate Naphthol AS-MX

1 x 40 mL

**PLATES** Disposable multi well (4 wells in each plate)

10

**COVER** in black color for the plates

1

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:** neutral red.

## 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.

**Timer**

**Deionized water**

## SAMPLE

**Blood (preferably from capillary) or bone marrow smears.** Blood samples may be collected with heparin. Avoid EDTA.

**STABILITY:** few days at room temperature and protected from dust. Fixed slides can be stored for many weeks.

## MANUAL ASSAY PROCEDURE

### A) FIXATION OF SLIDES (see notes)

- Fix the air-dried smears for 1 minute in the fixing solution.
- Wash both sides of the slide thoroughly in deionized water, drain, and wait for it to dry. The fixative contains formaldehyde. Even a small amount of formaldehyde on the slides can cause enzyme inhibition.

### B) PREPARATION OF WORKING SOLUTION

Bring the reagents to room temperature before use. Unscrew the cap of a vial of Reagent 1 and gently remove the rubber cap.

- Take 4 mL of Reagent 2 using a pipette or Pasteur pipette and add it to a bottle of Reagent 1. Replace the rubber cap and shake by inversion until the lyophilized reagent is completely dissolved.

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

### C) ALKALINE PHOSPHATASE REACTION

- Put the needed multi-well plates on a flat surface. Each plate and each bottle of working solution allows to run 4 determinations.
- 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.
- 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.
- Take 1 mL of working solution using a pipette or Pasteur pipette. Insert the tip of the pipette or Pasteur pipette into the central area of the groove and inject the working solution. Less than 1 mL is enough to fill the well. Proceed the same way with the other slides.
- Cover the well with the black cover. If more plates are used, put them one over the other before covering.

- Incubate for 30 minutes at room temperature (18-26°C).

- 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 wells can be used for the storage of slides.

## D) COUNTERSTAINING

- Counterstain in neutral red for 3 minutes.
- Rinse in running water, dry and read under the microscope. Experience in cytochemical techniques allows for the evaluation of slides without counterstaining.

## CALCULATION

A diffused blue color and the presence of bluish granules proves enzymatic activity. The cores are evident if contrast staining is used. To perform a quantitative determination, count 100 granulocytes and assign a point from 0 to 3 based on the color intensity on the number of granules in the cytoplasm.

| POINTS |  |
|--------|--|
| 0      | no pigmentation, absence of granules   |
| 1      | widespread pigmentation, scanty granules   |
| 2      | widespread stronger pigmentation, many granules  |
| 3      | strong pigmentation, cytoplasm crowded with a large amount of granules with an intense and uniform bluish pigmentation |

## RESULTS AND REFERENCE VALUES

See the following three points classification as an example:

| POINTS | x | number of cells | = | PAL QUANTITY |
|--------|---|-----------------|---|--------------|
| 0      | x | 65              | = | 0            |
| 1      | x | 20              | = | 20           |
| 2      | x | 10              | = | 20           |
| 3      | x | 5               | = | 15           |
|        |   | 100             |   | 55           |

The reference values commonly accepted varies from 20 to 120. Each laboratory should define its own reference values.

Positive values can be obtained from patients with pyogenic leucocytosis, from women in their third month of pregnancy or in the first days after giving birth.

## PATHOLOGY

Leucocyte alkaline phosphatase (PAL) is subject to several variations:

|  |                      |
|--|----------------------|
| Infections                                 | Increased            |
| Pregnancy                                  | Increased            |
| MYELOPROLIFERATIVE SYNDROMES               |                      |
| primitive polycythemia                     | normal or increased  |
| myelofibrosis in status phase              | increased or normal  |
| chronic myeloid leucosis                   | considerably reduced |
| MYELOYDPLASTIC SYNDROMES                   |                      |
| refractory anaemia with excess of blasts   | Reduced              |
| chronic myelomonocytic leucosis            | Reduced              |
| paroxysmal nocturnal hemoglobinuria        | considerably reduced |
| idiopathic primitive sideroblastic anaemia | normal or reduced    |
| PHARMACOLOGICAL TREATMENTS                 |                      |
| treatments with steroid hormones           | possible increase    |

## NOTES

Plates can be used for fixing and counterstaining the smear.

## 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          |

