SIGMA-ALDRICH®

ALKALINE PHOSPHATASE (AP), LEUKOCYTE

(Procedure No. 86)

INTENDED USE

Sigma-Aldrich Alkaline Phosphatase kits are intended for the histochemical semi-quantitative demonstration of alkaline phosphatase activity in leukocytes. Alkaline Phosphatase reagents are for "In Vitro Diagnostic Use".

In hematopoietic tissue, alkaline phosphatase appears restricted to band and segmented neutrophils.[1-2] Its demonstration by simultaneous capture using substituted naphthols and diazonium salts is, perhaps, the earliest example of a cytochemical enzyme test with clinical significance.[3]

Most procedures, including those provided by Sigma-Aldrich, employ stable diazonium salts. These are formed by reacting an arylamine with sodium nitrite in an acid medium.[4] The resulting diazonium chloride (usually unstable) can then be treated with compounds such as zinc chloride, zinc sulfate or naphthalene-1,6-disulfonate, forming stable salts. These stabilizers may exert marked inhibition on some enzymatic systems whereas the diazonium chlorides are less inhibitory.[4] For this reason, Sigma-Aldrich now provides stable solutions of fast red violet LB base, fast blue BB base, and sodium nitrite for alkaline phosphatase cytochemistry. To further simplify these methods, a stable solution of naphthol AS-BI phosphate is included.

To perform the test, fixed blood films are incubated at room temperature (18–26°C) in a solution containing naphthol AS-BI phosphate and freshly prepared fast red violet LB salt or fast blue BB salt buffered at pH 9.5 with 2-amino-2-methyl-1,3-propanediol (AMPD). Sites of activity are either red or blue depending upon choice of diazonium salt. The procedure incorporating fast red violet LB is similar to a proposed NCCLS reference method.[5]

REAGENTS

NAPHTHOL AS-BI ALKALINE SOLUTION, Catalog No. 861-10 ml
Naphthol AS-BI phosphate, 4 mg/ml, in AMPD buffer, 2 mol/l, pH 9.5.
FRV-ALKALINE SOLUTION, Catalog No. 862-10 ml
Fast red violet LB base, 5 mg/ml, in hydrochloric acid, 0.4 mol/l, with stabilizer.
FBB-ALKALINE SOLUTION, Catalog No. 863-10 ml
Fast blue BB base, 5 mg/ml, in hydrochloric acid, 0.4 mol/l, with stabilizer.
SODIUM NITRITE SOLUTION, Catalog No. 914-10 ml
Sodium nitrite, 0.1 mol/l.
CITRATE SOLUTION, Catalog No. 915-50 ml
Citric acid, 18 mmol/l, sodium citrate, 9 mmol/l, sodium chloride, 12 mmol/l with surfactant, buffered at pH 3.6.
HEMATOXYLIN SOLUTION, GILL NO. 3, Catalog No. GHS3-50 ml
Hematoxylin, certified, 6.0 g/l, sodium iodate, 0.6 g/l, and aluminum sulfate, 52.8 g/l, with stabilizers.
NEUTRAL RED SOLUTION, BUFFERED, Catalog No. N6264-50 ml
Neutral red, certified, 0.5% w/v, in acetate buffer, pH 5.2. Preservative added.

STORAGE AND STABILITY:
Store Naphthol AS-BI Alkaline Solution, FRV-Alkaline Solution, FBB-Alkaline Solution and Sodium Nitrite Solution in refrigerator (2–8°C). Reagents are stable until expiration date.
Store Citrate Solution in refrigerator (2–8°C). Solution is suitable for use in the absence of microbial growth.
Store Hematoxylin Solution and Neutral Red Solution at room temperature (18–26°C). Protect Hematoxylin Solution from light. Reagents are stable until expiration date.

DETERIORATION:
Discard Hematoxylin Solution if solution turns brown (over-oxidized from air) or purple (loss of acidity).

PREPARATION:
Reagents are provided ready for use.

CITRATE-ACETONE-FORMALDEHYDE FIXATIVE: To 25 ml Citrate Solution, Catalog No. 915-50 ml, add 65 ml Acetone and 8 ml of 37% Formaldehyde. Place in glass bottle and cap tightly. Store in refrigerator (2–8°C). Warm to 18–26°C prior to use. Stable up to 4 weeks if stored tightly capped in refrigerator.

PRECAUTIONS:
Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet and product labeling for any updated risk, hazard or safety information.

PROCEDURE

SPECIMEN COLLECTION:
It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Fresh blood or bone marrow films or samples anticoagulated with heparin may be used.[5] AVOID EDTA.[5] Blood smears should be stained for enzyme activity within 8 hours after preparation. However, if this is not possible, gradual loss of alkaline phosphatase activity may be delayed by fixation and storage overnight in freezer.[5] Films should be dried at least 1 hour prior to fixation and 3 hours post-fixation before freezing.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:
Acetone, ACS Reagent
Formaldehyde, 37% ACS

NOTES:
Perform procedure using positive controls. These can be obtained from patients with pyogenic leukocytosis, or women in the third trimester of pregnancy or during the first several days postpartum. Leukocyte alkaline phosphatase scores from these persons usually exceed 100. A negative control can be prepared from a normal fixed smear by immersing it in boiling water for 1 minute to inactivate the enzyme. Control films can be preserved up to 1 year if stored fixed, wrapped in Parafilm® at –70°C. These films should be dried at least 1 hour prior to fixation and 3 hours post-fixation before freezing.

It is strongly recommended that each laboratory establish its own expected range, characteristic for the local population.

The procedure depends upon subjective rating of stained cells. This can result in a wide variation of ratings obtained. The temperature of the reaction mixture must be kept between 18–26°C. Lower temperatures will result in significantly lower scores. Above 30°C, marked increases in activity will occur. Eosinophils do not stain but can be recognized by bilobed nuclei and large refractile granules.

There is a scarcity of data concerning compounds which may interfere with leukocyte alkaline phosphatase activity (LAPA). Certain drugs and other substances are known to influence circulating alkaline phosphatase activity.[6] Oral contraceptives, cortisol and stress may result in elevated leukocyte alkaline phosphatase scores.[3]

In humans, alkaline phosphatase activity is restricted to mature and band-form granulocytes. Occasionally weak staining may be observed in lymphocytes. Bone marrow osteoblasts and endothelial cells stain strongly. Marked increase in peripheral blood leukocyte alkaline phosphatase is observed in multiple myeloma, Hodgkin's disease, polycythemia vera and infectious leukocytosis. Low or absence of activity is seen in chronic myelocytic leukemia, hereditary hypophosphatasia and paroxysmal nocturnal hemoglobinuria.

The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnostic tests or information.

PROCEDURE
NOTE: For use with Columbia jars, divide reagent volumes by 5.

1. Measure 45 ml deionized water and adjust temperature to 18–26°C.
2. Prepare diazonium salt solution:
   Add 1 ml Sodium Nitrite Solution, to 1 ml of FRV-Alkaline Solution
   OR
   Add 1 ml Sodium Nitrite Solution, to 1 ml of FBB-Alkaline Solution.
   Mix by gentle inversion. Allow to stand for 2 minutes.
3. Add solution prepared in Step 2 to the deionized water from Step 1.
4. Add 1 ml Naphthol AS-BI Alkaline Solution, to diluted diazonium salt solution (Step 3). Mix thoroughly and pour into a Coplin jar.
5. Bring Citrate-Acetone-Formaldehyde Fixative to room temperature (18–26°C). Fix slides by immersing in fixative solution for 30 seconds. Rinse gently in deionized water for 45 seconds. Do not allow slides to dry.
6. Add slides to alkaline-dye mixture (Step 4) and incubate at 18–26°C for 15 minutes. Protect immersed slides from direct light. Discard alkaline-dye mixture after use.
7. After 15 minutes incubation, remove slides from Coplin jar and rinse for 2 minutes in deionized water. Do not allow slides to dry.
8. Counterstain for 2 minutes. If using FRV-Alkaline Solution, counterstain with Hematoxylin Solution, Gill No. 3. If using FBB-Alkaline Solution, counterstain with Neutral Red Solution, Buffered.
9. Rinse slides thoroughly in tap water and air dry.
10. Evaluate microscopically. If coverslipping is required use only an aqueous mounting media.

PERFORMANCE CHARACTERISTICS

METHOD OF SCORING:
Scan the film (900X) and select a thin area where erythrocytes are barely touching. Sites of phosphatase activity will appear as blue or red granules, depending upon dye used. Select 100 consecutive segmented and band-form neutrophilic granulocytes. Rate from 0 to 4+ on the basis of quantity and intensity of precipitated dye within the cytoplasm of these cells. For characteristics of scoring, refer to Table 1. The sum of the ratings of 100 cells is regarded as the score.

TABLE 1. CHARACTERISTICS OF SCORING*

	Precipitated Azo Dye in Cytoplasm			
Cell Rating	Amount** (%)	Size of Granule	Intensity of Staining	Background of Cytoplasma
0+	None	—	None	None
1+	50	Small	Faint to Moderate	Colorless to very pale pink or blue
2+	50–80	Small	Moderate to Strong	Colorless to pale pink or blue
3+	80–100	Medium to Large	Strong	Colorless to pink or blue
4+	100	Medium and Large	Brilliant	Not visible

* Table 1 represents modification of observations made by Kaplow.[2,3]
** Percentage of volume of cytoplasm occupied by azo dye precipitate.

To obtain the leukocyte alkaline phosphatase activity (LAPA) score, the number of cells counted is multiplied by the value for cell rating. These figures are added to obtain the LAPA score as shown in the following examples:

Cell Rating	Counted Score	LAPA Score
0	60	0
1+	20	20
2+	14	28
3+	5	15
4+	1	4
Total	100	67

The following scores from 40 healthy individuals were obtained at Sigma-Aldrich using the procedure described.

	Fast Red Violet LB	**Fast Blue BB**
LAPA Mean ± 1 SD	76±31	94±39
LAPA Range	44–106	55–133

The range of normal scores is wide, varying from 20 to 180. The following range of scores were obtained from those 40 normal individuals:

Fast Red Violet LB	**Fast Blue BB**
20–146	25–180

If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

REFERENCES

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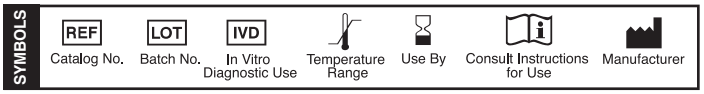

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